



Original Articles

Serological evidence and spatial analysis of small ruminant lentiviruses in herds in Maranhão, Brazil

Evidência sorológica e análise espacial de lentivírus em pequenos ruminantes no Maranhão, Brasil

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ABSTRACT

Caprine arthritis encephalitis and Maedi-Visna are lentiviruses affecting goats and sheep, respectively. Despite the literature having studies about these diseases, there is a constant demand and the need to study the health status of flocks that exploit economically. Therefore, this study aimed to assess the frequency of small ruminant lentiviruses explored in regional locations of Chapadinha and Itapecuru Mirim, that compose the microregion of Low Parnaíba, Maranhão, Brazil, as well as analyze the spatial distribution of outbreaks in the studied regions. Therefore, 241 properties were visited, where blood was collected in 1150 sheep and 1260 goats and tested by agar gel immunodiffusion (AGID). Epidemiological questionnaire was applied and collected the geographic coordinates. There was a low frequency for lentivirus, with 0.39% (5/1260) of goats and 0.08% (1/1150) of sheep. Regarding the spatial analysis, the reagent flocks were distributed in strategic cities for commercialization throughout the microregion. There was a low occurrence of lentiviruses. The municipalities of Cantanhede and Pirapemas of the regional of Itapecuru Mirim and Brejo and Magalhães de Almeida had reagent flocks for CAE. Whereas the municipality of Matões do Norte presented flock reagent to Maedi-Visna, this belonging to the regional of Chapadinha.

RESUMO

A artrite encefalite caprina e a Maedi-Visna são lentivírus que afetam cabras e ovelhas, respectivamente. Apesar da literatura apresentar estudos sobre essas doenças, existe uma demanda constante e a necessidade de se estudar o estado de saúde dos rebanhos explorados economicamente. Portanto, este estudo teve como objetivo avaliar a frequência de lentivírus de pequenos ruminantes explorados nas regionais de Chapadinha e Itapecuru Mirim, que compõem a microrregião do Baixo Parnaíba, Maranhão, Brasil, bem como analisar a distribuição espacial dos surtos nas regiões estudadas. Para tanto, foram visitadas 241 propriedades, onde foi coletado sangue de 1150 ovelhas e 1260 cabras e testado por imunodifusão em gel de ágar (IDGA). Foi aplicado questionário epidemiológico e coletado as coordenadas geográficas. Houve baixa frequência para lentivírus, com 0,39% (5/1260) de caprinos e 0,08% (1/1150) de ovinos. Quanto à análise espacial, os rebanhos reagentes foram distribuídos em cidades estratégicas para comercialização em toda a microrregião. Houve baixa ocorrência de lentivírus. Os municípios de Cantanhede e Pirapemas da regional de Itapecuru Mirim e Brejo e Magalhães de Almeida possuíam rebanhos reagentes para CAE. Já o município de Matões do Norte apresentou reagente rebanho à Maedi-Visna, esta pertencente à regional de Chapadinha.

Palavras-chave:

Artrite encefalite caprina

Georreferenciamento

Imunodifusão em Gel de Ágar

Maedi-Visna

Fator de Risco

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INTRODUCTION

The lentiviruses in small ruminants comprise the caprine arthritis encephalitis virus (CAEV) and Maedi-Visna (MVV), infecting goats and sheep respectively. They are RNA virus belonging to the family *Retroviridae*, Orthoretrovirinae subfamily, genus *Lentivirus* (MURRAY, 2000). These viruses cause different clinical manifestations, being in sheep chronic pneumonia and encephalitis (MVV) and caprine arthritis and leucoencephalomyelitis (LIMA et al., 2018).

In infected flocks, the virus elimination occurs mainly by means of secretions, more specifically, defense cells containing viral particles. The transmission occurs by the animal's direct contact, especially in intensive creations (KALOGIANNI et al., 2020).

The goat/sheep production is a shepherd activity present in all the continents (LOBO, 2019). Found in the most diverse ecosystems, with different climate and vegetation, it is exercised not only in regions with plenty of water and food but also in semi-arid areas. However, only in some countries, this activity has significant economic expression, although many of them are still practiced without technical guidance and with low productivity and compromised profitability (MOURÃO et al., 2016; NOGUEIRA FILHO; ALVES, 2002).

The goat and sheep flocks are numerically significant in the Northeast. And in Maranhão has a squad of 250,871 and 193,141 animals, respectively (IBGE, 2017). However, these activities have a character of subsistence, being practiced mostly by low-income farmers, managed extensively or even ultra-extensive, with low profitability and mainly, with poor or no veterinary care (PEREIRA et al., 2018).

The implantation of adequate management to regional conditions, addressing the nutritional and sanitary factors, and the adoption of adaptive technologies, are relevant to sustainable development and to promote the quality of life of man in the field. When it follows an efficient program of animal herds is not followed, they become susceptible to diseases of various etiologies, among which we can mention - Maedi Visna (MV) and the Caprine arthritis encephalitis (CAEV) (GOMEZ-LUCIA; BARQUERO; DOMENECH, 2018; SARDI et al., 2012).

For the detection of these viral agents, the Agar Gel immunodiffusion (AGID) is used, serological technique recommended by the International Organization for Animal Health (OIE), because of its low cost, easy applicability, good specificity and sensitivity and for being reasonable and being indicated as a screening test in the programs of lentiviruses. Considering the triad: "animal health, food production and health", the importance of these activities and the fact that no work was performed at the microregion of the Low Parnaíba, Maranhão, the present work aimed to verify the

occurrence of lentiviruses in goat and sheep flocks and study their spatial distribution.

MATERIAL AND METHODS

Between January and March 2015, 126 flocks were chosen randomly, without any specific criteria, that exploit goats and 115 that exploit sheep, distributed in the municipalities that compose the Regionals of Chapadinha and Itapecuru Mirim, Maranhão: Água Doce, Anapurus, Araisos, Brejo, Buriti, Chapadinha, Magalhães de Almeida, Paulino Neves, Santa Quitéria, São Bernardo, Tutóia, Urbano Santos, São Benedito do Rio Preto, Anajatuba, Cantanhede, Itapecuru Mirim, Matões do Norte, Miranda do Norte, Pirapemas, Presidente Vargas, Santa Rita and Vargem Grande. These regional concentrate most of the goats and sheep of the State, even facing adverse conditions such as low rainfall and little green vegetation. Most of the animals are handled in an ultra-extensive manner, without regard to sanitary and food conditions, leaving them exposed to several risk factors.

Of each herd 10 samples of blood serum were collected, making a total of 1260 and 1150 samples of goats and sheep, respectively. This sample was obtained by means of the formula of Callegari-Jacques (2013) with a sampling error of 8% and following the principle of proportionality of the population of animals and herds for each municipality. The animals were randomly chosen, regardless of age, race and sex.

The samples were collected from the venipuncture of the external jugular vein, previously sanitized with iodine solution to 10% in 10mL tubes and transported to the Laboratory of Infectious Diseases of the State University of Maranhão. They were centrifuged at 2500 g/5 minutes and subsequently placed in polyethylene tubes, identified with the number of the animal and of the flock and stored at -20°C. For each flock, an epidemiological questionnaire was applied to evaluate possible situations associated with the disease, such as: concentration, clinical signs, animal transit guide, consortium creation, type of exploration, breeding and participation in events.

The sera were subjected to the test of agar gel immunodiffusion (AGID), according to the rules of OIE (2011) for the diagnosis of CAE and MV, through commercial reagent kit of LTDA - ME (Bioveteck Kit®, Recife, PE). This kit contains protein antigen (p28) extracted from the virus of CAE. After the disposal of the sera suspects, of positive controls and of the antigen in their respective holes, the slides were incubated in a humid atmosphere at a temperature between 20°C and 25°C, with the reading performed after 48 hours of incubation. The presence of the line formed between the antigen and positive control serum is the basis for the reading of the test and indicates the presence of antibodies in the tested serum samples and, consequently, the classification of the animal as reagent.

For the spatial study a receiver of the Global Positioning System - GPS (TrackerMultilaser®) Garmin® brand was used with an accuracy of approximately two meters, for geographical coordinates of the municipalities and the herds sampled, related in latitudes and longitudes, with its system in degrees, minutes and seconds. To obtain the maps the QGIS 2.2 software was used.

The results of frequency of infection in the evaluated municipalities were calculated by dividing the number of positive animals by the number of animals sampled, using simple descriptive statistical analysis and by means of absolute and relative distributions (SAMPAIO, 2007).

This study was approved by the Committee on Ethics and Animal Experimentation (CEEA/CVMP/UEMA), according to process no. 041/2014.

RESULTS

In accordance with the analyzes of the AGID test, the frequency of reagent animals was 0.24% (6/2410). In relation to the species it was observed that 0.39% (5/1260) of goats and 0.08% (1/1150) of sheep were reagent (Table 1).

Table 1. Frequency of animal's reagent to Lentivirus in the IDGA test, in Regionals of Chapadinha and Itapecuru Mirim, MA, 2015.

Species	IDGA		Regional	
	Total tested	Positive	Itapecuru Mirim	Chapadinha
Goats	1260	5 (0.39%)	3/530 (0.57%)	2/730 (0.27%)
Sheep	1150	1 (0.08%)	1/550 (0.18%)	0/600 (0%)
Total	2410	6 (0.24%)	4/1080 (0.37%)	2/1330 (0.15%)

The reagent goat flocks belonged to the municipalities of Brejo and Magalhães de Almeida, belonging to the regional of Chapadinha, as shown in Table 2, which shows the list of municipalities, number of effective herds, sampled herds and reactive herds. The same can

be seen in Table 3, with emphasis on the municipalities of Cantanhede, Matões do Norte and Pirapemas, of the Regional Itapecuru Mirim with reactive sheep herds (Tables 2 and 3).

Table 2. Distribution of frequencies of infection by CAE in caprine herds in the municipalities of the Regionals of Chapadinha and Itapecuru Mirim - Maranhão, 2015.

Regional	Municipality	No of effective herds goats	No of samples herds goats	No reagent flocks
Chapadinha	Água Doce	54	7	0
	Anapurus	13	2	0
	Araioses	72	9	0
	Brejo	43	5	(1/5) 20%
	Buriti	19	2	0
	Chapadinha	58	7	0
	Magalhães de Almeida	173	22	(1/22) 4.45%
	Paulino Neves	32	4	0
	Santa Quitéria	52	6	0
	São Bernardo	36	4	0
	São Benedito do Rio Preto	22	2	0
	Tutoia	5	1	0
	Urbano Santos	20	2	0
Total		599	73	2
Itapecuru Mirim	Anajatuba	39	8	0

Cantanhede	27	6	(2/6) 33.4%
Itapecuru Mirim	30	6	0
Matões do Norte	7	1	0
Miranda do Norte	16	3	0
Pirapemas	46	10	(1/10) 10%
Presidente Vargas	4	1	0
Santa Rita	15	3	0
Vargem Grande	71	15	0
Total	255	53	3

Table 3. Distribution of frequencies of infection by MaediVisna in sheep herds in the municipalities of the Regionals of Chapadina and Itapecuru Mirim - Maranhão, 2015.

Regional	Municipality	No existing sheep flocks	No samples sheep flocks	No reagent sheep flocks
Chapadina	Água Doce	49	3	0
	Anapurus	59	3	0
	Araioses	48	3	0
	Brejo	66	4	0
	Buriti	67	3	0
	Chapadina	132	14	0
	Magalhães de Almeida	223	13	0
	Paulino Neves	38	2	0
	Santa Quitéria	37	3	0
	São Bernardo	74	11	0
	Tutoia	7	1	0
Total	800	60	0	
Itapecuru Mirim	Anajatuba	35	3	0
	Cantanhede	58	2	0
	Itapecuru Mirim	33	3	0
	Matões do Norte	21	2	(1/2) 50%
	Miranda do Norte	14	3	0
	Nina Rodrigues	77	5	0
	Pirapemas	270	23	0
	Presidente Vargas	14	3	0
	Santa Rita	16	1	0
	Vargem Grande	118	10	0
Total	656	55	1	

Figures 1 and 2 show the spatial distribution of the reagent herds for CAE, in the municipalities of Catanhede and Pirapemas, belonging to Regional of Itapecuru Mirim

and Brejo do Maranhão and Magalhães de Almeida located in the Regional of Chapadina. And to Maedi Visna the municipality of Matões do Norte.

Figure 1. Georeferencing of outbreaks in flocks of goats in the Regionals of Chapadina and Itapecuru Mirim - regarding the diagnosis of CAE, 2015.

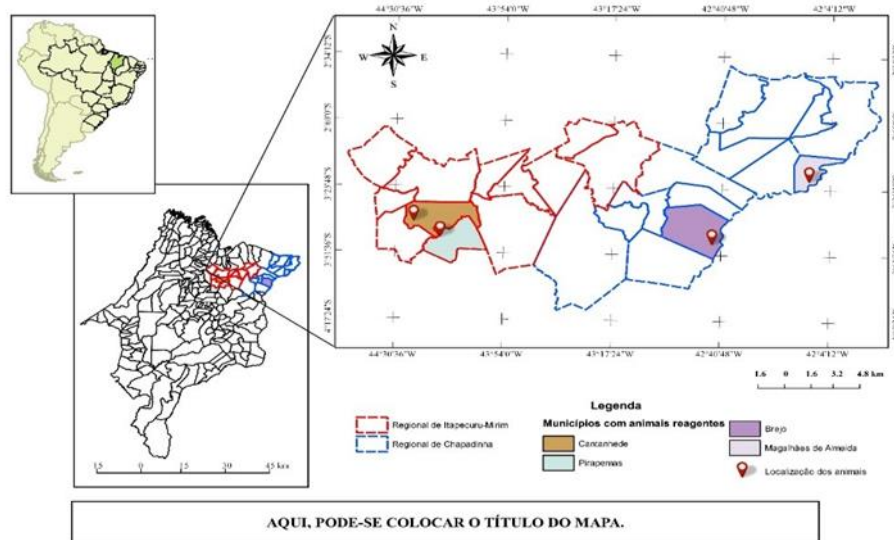
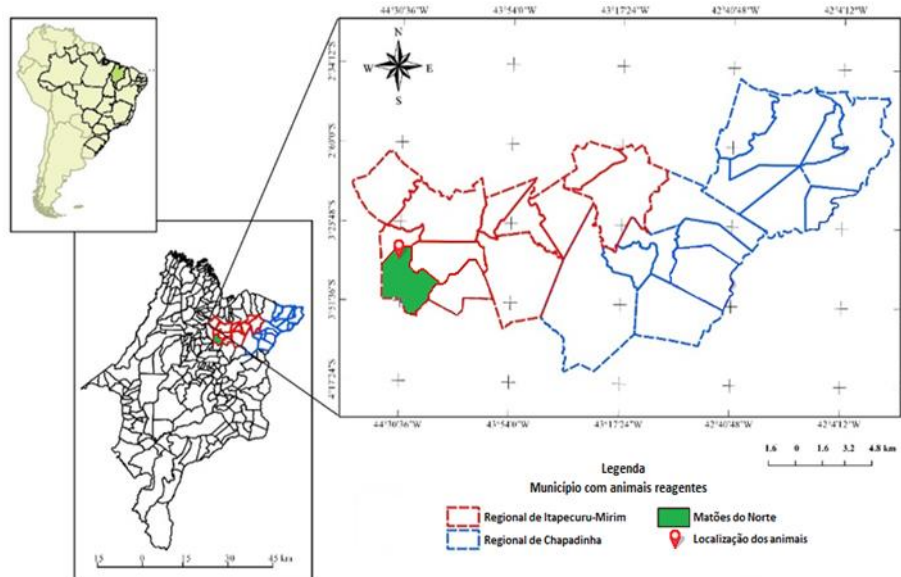


Figure 2. Georeferencing of outbreaks in flock of sheep in the Regionals of Chapadina and Itapecuru Mirim - regarding the diagnosis of Maedi Visna, 2015.



DISCUSSION

The low frequency found may be related to the low concentration of animals, but which could indicate that the infection by lentivirus may be widely distributed, but was not detected more frequently due to the characteristics of the type of breeding system adopted in those flocks, where the sanitary management is not practiced in a preventive manner (SOUSA et al., 2019). Several studies indicate that the environment and management have a fundamental role in the disease epidemiology and emphasize that the animal's agglomeration may facilitate the transmission. In contrast, in flocks with extensive system of creation, the prevalence is lower (MARTINEZ et al., 2011). Similar results were identified in other states of the Brazilian Northeast: 2.6% in Ceará, 16.2% in Rio Grande do Norte, 5% in Paraíba (ALVES et al., 2018), 0.5 and 0.3% in Bahia

(MARTINEZ et al., 2010; SOUZA et al., 2007), and 2.77% in Sergipe (RIZZO et al., 2018).

In a study carried out in the mesoregion of West of Maranhão State (MOURÃO et al., 2016), found the frequency of 7.5% (53/710), with 12 of the 14 municipalities evaluated reagent for lentivirus and animals presenting clinical manifestations such as lymphadenitis, mastitis, pneumonia and arthritis. However, it is not clear in the study why such high frequency in that flock, once all conditions of the flock are similar to those described in this study and others performed in the states of Northeastern Brazil. One way out of this understanding is that the manifested clinical signs are not pathognomonic, since other diseases may show the same signs, such as aspiration pneumonia or bacterial origin, arthritis by *Mycoplasma* spp, mastitis of bacterial origin and lymphadenitis caused by

Corynebacterium pseudotuberculosis (PRITCHARD; MCCONNELL, 2007)

Another thing to be considered is the requirement of non-reagent animals for lentivirus by the creators when purchasing them. This fact was confirmed by Mazzinghy et al. (2016), since the achievement of seronegative results and with animal transit guide is an effective preventive measure against the introduction of new cases in the state of Tocantins, place of work, since that, regions in increase stage of its herds end up purchasing a large amount of animals, subject to greater risk of purchasing animals with diseases, as well as facilitating the tracing of the animal and knowing with precision their origin. However, the search for negative herds is a very wide bias, a consequence of a lack of communication between breeders and technical assistance and by the few laboratories that carry out this test, being mostly available in the form of agar gel immunodiffusion kits.

In the study developed in Costa Risca, Villagra-Blanco et al. (2015) showed that 98 properties (71%) tend to separate the animals in paddocks, respecting age and clinical conditions. Even this attention does not detract from the fact that animals were exposed to the infection risks by the virus, once they found the frequency of 1.95%. Another situation that can be extrapolated is the consortium creation, which can be a favorable factor for the transmission and maintenance of lentivirus in herds. It was observed that, among the 6 reactive herds, one presented mixed breeding of sheep and goats, that is, two species grazing in the common environment and being accommodated in the same fold. Olech, Valas and Kuźmak (2018) stated that sheep and goats can be infected by a variety of lentivirus subtypes and some animals can be co-infected with viruses belonging to different subtypes. In addition, lentiviruses that infect goats and sheep follow a pattern of genomic evolution similar to that seen in HIV or other lentiviruses. Contamination between species is possible, between sheep and goats, in close contact and that horizontal transmission can occur naturally (CAROLINE; MINARDI; JEAN-FRANÇOIS, 2010).

Another point that should be dealt with the type of exploitation, whether meat, milk, or both. Stonos et al. (2013) found a prevalence of 80.4% in the dairy sector, identifying the lentivirus as a major health problem. In properties that exploit milk and dairy the animals are kept for a longer time and reared together, exposing the greater risk of maintenance of the agent in the site. The exploitation of milk in Maranhão, more specifically in the regional worked, is very low, the most serving as a supplement in the diet. Another factor that can be assigned is the late seroconversion, characteristic of infections by Lentiviruses, which may also have been a determinant factor in the frequency found at work, because they could not be identified in the IDGA test technique and thus reflected in low frequency. The animals intended for slaughter have an average age of 11 months, which hinders the spread of the virus, as well as

clinical manifestation of possible carriers. Tageldin et al. (2011) confirmed this fact when evaluating goats in the Sultanate of Oman. The low occurrences of illness are also associated with the short time of permanence of animals in flocks, being intended for own consumption, sale or marketed with other creators. According to Kalogianni et al. (2020), the type of exploration appears as a risk factor for dissemination of the agent. It is understood that the less time an animal remains on a property, the less the chances of it being infected and, if it is, not showing clinical signs or other manifestations, such as a drop in meat and milk production, as they tend to be belatedly (GOMEZ-LUCIA; BARQUERO; DOMENECH, 2018).

Sardi et al. (2012) in a study conducted in the Bahia state semi-arid found no positive animals in 1750 samples of goats and sheep using the IDGA test, where 59.8% of the properties practiced extensive management. The predominance of the extensive system of creation, associated with inadequate management practices were limiting factors to the development of sheep production in the region and may be related to the presence of the virus in flocks. As it was observed in the present study, there was the predominance of the extensive system in the evaluated flocks, in which the animals spend the day grazing in native grasslands and only return in the late afternoon to the property. This information may have interfered with the frequency found, since the concentration of a large number of animals in closed environments and with poor ventilation favors the spread of the virus.

In this study, 62.5% of the flocks were Santa Inês breed and without defined breed (SRD). It should be noted that to the extent that a flock passes through a breeding process, both goat and sheep, there is a trend of increasing prevalence of diseases, such as the lentiviruses (TEIXEIRA et al., 2016a; TEIXEIRA et al., 2016b). In the present study it was observed a predominance of SRD animals and without zootechnical bookkeeping, which may have influenced the low prevalence found. It is not well explained yet, but the racial factor can be determinant for the higher incidence of the disease in the herd. One of these hypotheses is related to animals originating in a country with no history of the disease and which, when contacted, has no immune memory to overcome the infection (GOMEZ-LUCIA; BARQUERO; DOMENECH, 2018).

In the state of Maranhão, the health documentation required for transit of goats and sheep comprises the Health Attestation (MARANHÃO, 2019) and the Animal Transit Guide (GTA). When analyzing the six properties that had at least one animal reactive for lentivirus, it turns out that only one performs (1/6) performed the serological test to lentiviruses sporadically. In the state of Maranhão, the test is not compulsory, but is indicated by the Ministry of Agriculture, Livestock and Food Supply (MAPA), demanding health care by the creator, so that there is not the introduction and spread of infectious agents. Whatever the purpose of movement of

animals, the requirement for health attestation must be preceded, followed by quarantine and realization of retests in the origin property (SOUSA et al., 2019; SARDI et al., 2012).

Related to participation in agricultural events, only one of the six properties participated, even so, it is important to consider that events such as these promote the direct contact of goats with other animals, including sheep, which increases the likelihood of transmission of certain pathogens. In these events usually there is the purchase and sale of animals, where many creators acquire breeders and/or matrices aiming at the improvement of their flock. The commercialization of animals is considered a risk factor of relevance in the dissemination of CAEV (SINGH; SINGH; KUMARI, 2018), being essential the control of the transit of animals in order to minimize the virus infection in the flocks. In an opposite manner to found by Tavella et al. (2018), the variable completion of quarantine was not a protection factor in the seropositive properties, since not all of them have the habit of doing it due in large part to the system of creation that does not provide a greater costing of the flock.

The analysis of the maps indicated a differentiated cluster center between the municipalities of Matões do Norte, Pirapemas and Cantanhede. These municipalities, as well as Brejo and Magalhães de Almeida, concentrate large numbers of sheep and goats and can be considered strategic locations for the purchase and sale of small ruminants, which can contribute to diseases transmission to neighboring municipalities, and may reach across the state and even neighboring states such as Piauí, Pará and Tocantins. The greater the population density, the greater the high frequency contact, contributing to the greater spread of the virus. As proposed by Carrozza et al. (2018), the spread of these viruses could have been enabled by the creation of new contact networks in livestock populations, leading to particular small ruminant breeds and populations being brought into contact for the first time. Certain limitations were imposed on the present study, such as the absence of published data on the frequency of the diseases assessed. However, even with an analysis of the serological results, it was possible to establish, although in a simple way, a model for the incidence of these viruses. Even though shy, it is still a contribution, mainly for future works that will have a previous basis. A focus must be kept on future works, aiming now at the spatial dynamics contrasting factors such as climate, humidity and population structure

Other studies should be carried out regarding better understand the occurrence of lentiviruses in these flocks. Diagnostic techniques with higher sensitivity and specificity must be used, due to the reason of the importance that the sheep has for the state of Maranhão.

CONCLUSIONS

In the flocks of the microregion of Low Parnaíba of Maranhão state, there was a low occurrence of lentiviruses. The municipalities of Cantanhede and Pirapemas of the regional of Itapecuru Mirim and Brejo and Magalhães de Almeida had reagent flocks for CAE. Whereas the municipality of Matões do Norte presented flock reagent to MaediVisna, this belonging to the regional of Chapadinha.

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