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*Original Article*

# Another case of sheep-associated malignant catarrhal fever in Rio Grande do Sul: the curious epidemiological niche of ovine gammaherpesvirus 2 in Southern Brazil

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## Abstract

Introduction: Ovine gammaherpesvirus 2 (OvGHV2) is the cause of sheep-associated malignant catarrhal fever (SA-MCF). Although SA-MCF is endemic in most geographical regions of Brazil, outbreaks are more frequently reported in the State of Rio Grande do Sul (RS). This report describes the findings of an SA-MCF outbreak in cattle from RS and investigates the possible reasons for the elevated occurrence of outbreaks in cattle from this geographical region of Brazil.

Methodology: An outbreak of SA-MCF involving a 7-month-old dairy calf died acutely after presenting fever, profuse salivation, and respiratory difficulties. This calf was maintained on a farm that contained two asymptomatic sheep. A qPCR assay detected OvGHV2 DNA in multiple organs of the dead calf and blood from the two asymptomatic sheep.

Results: These findings confirmed OvGHV2-related infection in the calf and the participation of the two sheep as possible disseminators of this infection. A sheep:cattle ratio (SCR) that evaluated the chances of SA-MCF outbreaks to occur within the mesoregions of RS where cattle and sheep are reared simultaneously, demonstrated that geographical regions with an SCR > 0.15 have a significantly increased chance to develop outbreaks of SA-MCF in cattle relative to mesoregions with an SCR < 0.15 within RS.

Conclusions: The SCR may be used as a possible indicator for the occurrence of SA-MCF outbreaks in cattle within the state of Rio Grande do Sul. Additionally, the traditional sheep production system and rearing practiced within RS seem to favor the development of SA-MCF outbreaks in susceptible cattle populations.

**Key words:** disease outbreaks; epidemiology; *Macavirus*; sheep:cattle ratio.

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## Introduction

Ovine gammaherpesvirus 2 (OvGHV2) is the cause of sheep-associated malignant catarrhal fever (SA-MCF), a frequently fatal, viral infectious disease of ungulates [1-4]. OvGHV2 (Macavirus ovinegamma2) is a member of the Macavirus genus, subfamily Gammaherpesvirinae, family Orthoherpesviridae [5]. Furthermore, the genus Macavirus contains several members of veterinary importance, some of which have known pathogenesis in the development of malignant catarrhal fever (MCF), including alcelaphine gammaherpesvirus 1 and 2 (AlGHV1 and -2), and caprine gammaherpesvirus 2 (CpGHV2) [1,2,6]. Alternatively, there are some established Macavirus that have been identified in animals, but their exact role in the development of disease remains controversial, these include bovine gammaherpesvirus 6, BoGHV6 [7], and suid gammaherpesvirus 3, -4, and -5 [8].

Sheep are the asymptomatic reservoirs for OvGHV2, with typical clinical manifestations of SA-MCF occurring in a wide range of susceptible domestic and wild animals worldwide [2,4,9]. Recently, the list of mammalians infected by OvGHV2 was increased due to the molecular detection in free-ranging wild boars (*Sus scrofa*) from Southern Brazil [10,11] and in the southern pudus (*Pudu puda*) from Chile [12]. The classical forms of SA-MCF described in ungulates worldwide include the head-and-eye [1,9,13], alimentary [13,14], neurological [13], and cutaneous [15] manifestations. However, these classical forms are not distinct, since overlapping of these symptoms can be observed in the same infected animal [13]. The head-and-eye form is probably the most frequently observed in outbreaks of SA-MCF [9,13], being characterized by clinical manifestations including elevated fever (41-41.5°C), profuse mucopurulent nasal discharge, corneal edema, ulcerations of the oral cavity, and widespread lymph node enlargement [1,14]. Most outbreaks of SA-MCF are sporadic, with only a single or few animals in a herd being affected [4,13].

There are several descriptions of outbreaks of SA-MCF with and without direct contact with sheep [1,2]. Additionally, few studies have confirmed spontaneous infections of OvGHV2 in the asymptomatic host worldwide [16-20]. Furthermore, during most outbreaks of SA-MCF, sheep maintained on the farm are not frequently evaluated for the possible detection of OvGHV2. The objectives of this study were to present the results of an outbreak of SA-MCF from a herd of dairy cows maintained on a farm that contained sheep and explore the curious epidemiology of SA-MCF in the State of Rio Grande do Sul, Southern Brazil.

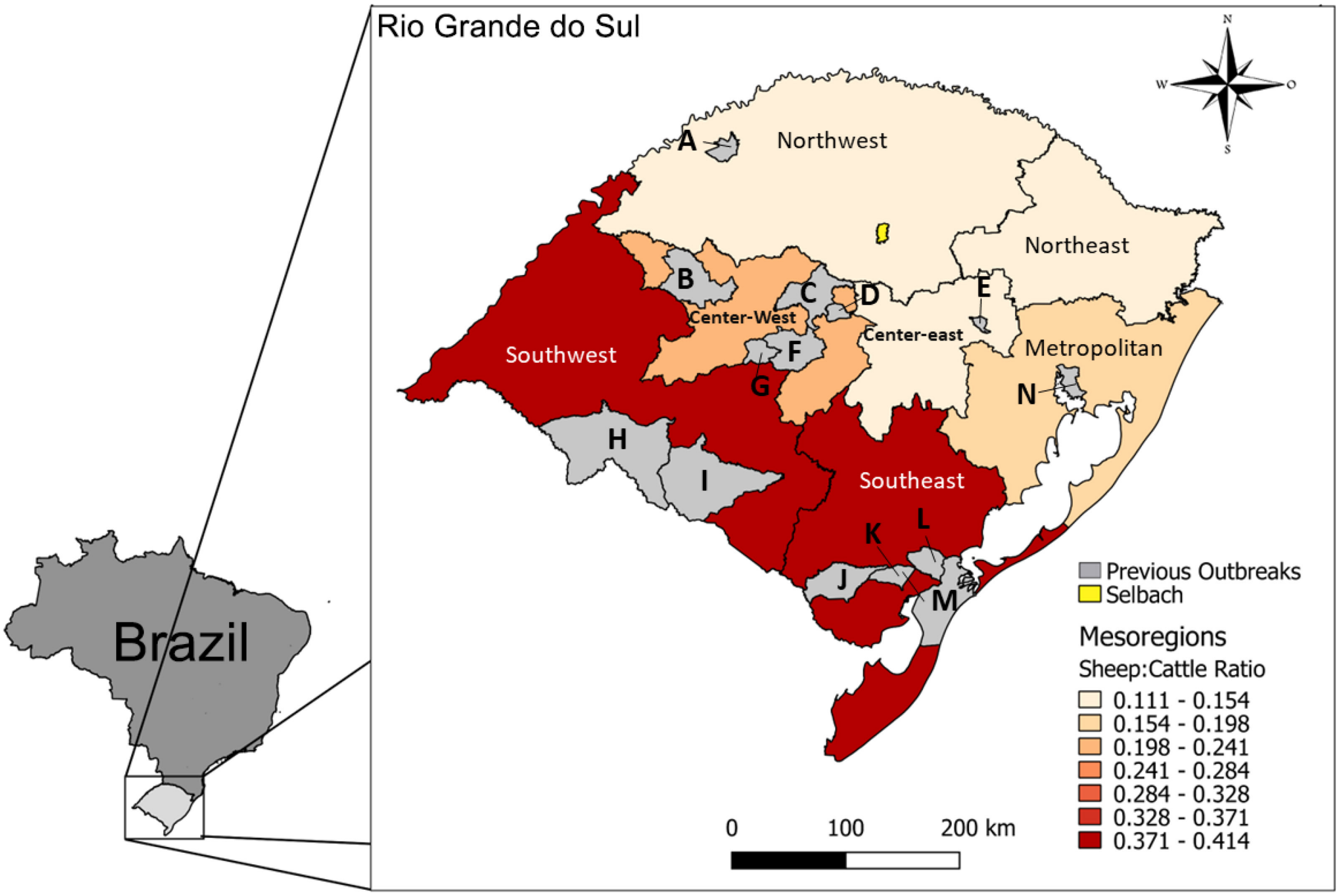
## Methodology

### *Study location and sampling*

A consulting veterinarian was summoned to a small subsistence dairy farm located on the outskirts of the municipality of Selbach due to the reported occurrence of excessive salivation and the loss of appetite in a 7-month-old calf. Selbach is a small municipality (176,471 km$^2$) located within the Northwest mesoregion of the State of Rio Grande do Sul (RS), Southern Brazil. The city has a population of 5,107 habitants, a Municipal Human Development Index (MHDI) of 0.777, as compared to an average MHDI of 0.771 in the RS [21], and contains 8,073 heads of cattle and 1,067 sheep [22].

The consulting veterinarian examined the calf and medicated the animal accordingly. By the end of the same week, the clinical status of the animal deteriorated, pneumonia was now the main suspected clinical disease, and the consulting veterinarian contacted the Regional Official Veterinary Service to

**Figure 1.** Graphical representation of the association between the sheep/cattle ratio in areas known to have outbreaks of sheep associated-malignant catarrhal fever in cattle within the mesoregions of the State of Rio Grande do Sul, Southern Brazil.



A: Santa Rosa; B: Santiago; C: Júlio de Castilhos; D: Nova Palma; E: Cruzeiro do Sul; F: Santa Maria; G: Dilermando de Aguiar; H: Sant'Ana do Livramento; I: Dom Pedrito; J: Herval; K: Pedro Osório; L: Capão do Leão; M: Rio Grande; N: Porto Alegre.

investigate the cause of the sudden illness. However, the Official Veterinarian found the calf dead on arrival at the farm.

This calf was maintained on a farm that contained two sheep (one 18-month-old male and a 2-year-old ewe) and 46 dairy cattle. Only the 7-month-old calf was affected, all other cattle did not present any clinical manifestation. Since SA-MCF was suspected, selected samples (kidney, eye, retropharyngeal lymph node, carotid rete mirabile (CRM), and cerebrum) were collected, placed on ice, and submitted to the Official Veterinary Diagnostic Laboratory. Additionally, whole blood from the two asymptomatic sheep, that co-mingled with the calf, was also collected for laboratory evaluation.

### *Molecular detection of ovine gammaherpesvirus 2*

The samples received were routinely processed at the Official Diagnostic Laboratory of Viral Diseases, Ministry of Agriculture and Livestock (MAPA), Minas Gerais, Brazil. For diagnostic purposes, the samples were pooled (pools #1-3; Table 1) and then used in qPCR assays designed to amplify the glycoprotein B (gB) gene of OvGHV2 as described [23]. Positive and negative controls were included in each run. A sample was considered positive when a quantification cycle (*Cq*) less than 33 was detected by the qPCR assays.

### *Estimation of SA-MCF outbreaks in cattle from Rio Gande do Sul*

Since sheep are the reservoir hosts for OvGHV2 [2,9], a possible relationship between sheep and cattle reared at each location in RS was obtained based on the published data of SA-MCF within this state [1,24]. These two studies were chosen since they collectively contain all known published outbreaks of SA-MCF in RS. This relationship was coined as the sheep:cattle ratio (SCR) and correlates the number of sheep and cattle reared concomitantly within the same geographical area. Input data to estimate the SCR from the municipality of the current outbreak (Selbach), as well as other municipalities within RS with reported published outbreaks of SA-MCF, was obtained from the database of the Brazilian Institute of Geography and Statistics (IBGE) using data collected for 2022 [22]. Consequently, the possible association between the SCR (cut-off, 0.15) and previous occurrences of SA-MCF outbreaks recorded in the State of RS was performed using the Chi-square test.

The association magnitude was determined by the odds ratio (OR); all calculations were performed in the OpenEpi™ software, version 3.01 [25], with a statistical significance of 5%. The data obtained was then plotted on a map of the State of RS generated with the qGIS software v2.18.14.

## Results

### *Epidemiological, clinical, and pathological findings*

The collected epidemiological data revealed that morbidity and mortality were extremely reduced (2.1%; 1/47), with elevated lethality (100%; 1/1). The clinical evaluation done by the consulting veterinarian confirmed profuse salivation, respiratory discomfort, and fever (40°C). Since the animal was found dead by the Official Regional Veterinary Service, tissues were not submitted for histopathological evaluation.

### *Quantification of OvGHV2 DNA by qPCR*

The results of the *Cq* detected in the pooled organs and biological samples relative to the positive control are provided in Table 1; OvGHV2 DNA was quantified from all samples with positive results since the *Cq* identified was < 33. Furthermore, the pooled samples derived from the calf with clinical manifestations suggestive of SA-MCF revealed a more elevated *Cq* (pool #1, 24.49; pool #2, 27.52) as compared to the asymptomatic sheep (pool #3, 30.91), indicating that the viral load detected in the tissues of the dead symptomatic calf was comparatively more elevated than that identified in the two asymptomatic sheep.

### *Effects of the sheep/cattle ratio on the occurrence of SA-MCF outbreaks in cattle from Rio Grande do Sul*

The dynamics of the outbreaks of SA-MCF are graphically demonstrated in Figure 1; where the risk of outbreaks of SA-MCF occurring within the State of RS increased proportionally with the increased SCR. Although sheep rearing occurs in all mesoregions of the State, most recorded outbreaks were predominant within municipalities located in the Southwestern and

**Table 1.** Quantification cycle (*Cq*) detected in the pool of organs and biological samples derived from a symptomatic calf and asymptomatic sheep by qPCR assay to detect the OvGHV2 glycoprotein gene.

| Pool # | Clinical status | Polled organs | Cq |
|---|---|---|---|
| 1 | Symptomatic calf | Kidney, eye, and retropharyngeal lymph node | 24.49 |
| 2 | Symptomatic calf | Brain and carotid rete mirabile | 27.52 |
| 3 | Asymptomatic sheep (n, 2) | Whole blood | 30.91 |
| 4 |  | Positive control | 18.75 |

Southeastern mesoregions where the SCR is > 0.328, and to some extent the Center western mesoregion. Collectively, these mesoregions constitute the largest geographical zone of the Pampa biome in RS [26]. Additionally, the risk of having outbreaks of SA-MCF within the Center Eastern, Northwestern, and Northwestern mesoregion of RS was comparatively reduced.

Furthermore, the SCR demonstrated that municipalities with a calculated ratio > 0.15 were more likely to have an outbreak of SA-MCF, as compared to those with a ratio less than 0.15 (Table 2). Contrastingly, four municipalities (Selbach, Cruzeiro do Sul, Rio Grande, and Santa Rosa) where outbreaks were registered within the State of RS had reduced chances of having outbreaks of SA-MCF. Of these four locations, only Rio Grande is situated within a mesoregion with elevated SCR. The current outbreak occurred in the Northwestern mesoregion of RS where the risk of a SA-MCF outbreak at this specific farm was relatively low, having a SCR of 0.04255 (2 sheep/47 cattle). Additionally, the municipalities of Santa Rosa and Cruzeiro do Sul are located within mesoregions with reduced SCR. Furthermore, the SCR detected within the municipalities of Selbach and Cruzeiro do Sul is relatively close to the cut-off limit.

## Discussion

The clinical manifestations of fever and excessive salivation observed in this calf are typical signs observed in cattle with the head-and-eye form of SA-MCF [1,2,9]. Additionally, the quantification of OvGHV2 from pooled samples derived from this calf confirmed that this animal was infected by this virus. It must be highlighted that the CRM contained in pool #2 is the organ of choice for the diagnosis of SA-MCF [1,27]. Furthermore, the positive quantification of OvGHV2 from biological samples of the two sheep maintained on the same farm demonstrated that these asymptomatic reservoir hosts were the most likely sources of infection; similar quantification results of OvGHV2 DNA were described in an outbreak of SA-MCF in cattle and sheep from Cruzeiro do Sul, RS [23] and in an asymptomatic goat from Paraná [28]. In addition, these findings are in accord with the known method of dissemination of SA-MCF [1,2,9], where asymptomatic sheep are the source of infection in susceptible animal populations. However, in several outbreaks of SA-MCF in other geographical regions of Brazil, including São Paulo [29], Minas Gerais [30,31], and Paraná [32,33], sheep were not reared concomitantly with the infected cattle or within close proximity. Interestingly, there is a report of SA-MCF in bison from the USA without any contact with sheep, where it was postulated that under special conditions aerosol transmission of OvGHV2 with subsequent infection can occur as far as 5 km distance between asymptomatic sheep and the susceptible animal [34]. Nevertheless, the participation of aerosol dissemination in the active transmission of OvGHV2 has not been demonstrated experimentally, so the possible role of other animal species or birds cannot be excluded. The

**Table 2.** Association between known outbreaks of SA-MCF in cattle and the sheep:cattle ratio within municipalities from the State of Rio Grande do Sul, Brazil.

| Sheep:cattle ratio | Associated factors for SA-MCF outbreaks | | Odds Ratio | CI (95) | p |
|---|---|---|---|---|---|
| | Outbreak | No outbreak | | | |
| > 0.15 | 11 | 194 | 4.536 | 1.425 - 14.44 | 0.01173 |
| < 0.15 | 4 | 320 | | | |
| Total | 15 | 514 | | | |

| Locations of SA-MCF outbreaks [1] | Animal populations [2] | | Sheep:cattle ratio |
|---|---|---|---|
| | Cattle (heads) | Sheep (heads) | |
| Selbach | 8,073 | 1,067 | 0.13217 |
| Capão do Leão | 31,843 | 7,776 | 0.24421 |
| Cruzeiro do Sul | 9,054 | 1,083 | 0.11962 |
| Dilermando de Aguiar | 42,208 | 10,042 | 0.23792 |
| Dom Pedrito | 3,33,050 | 1,11,886 | 0.33594 |
| Herval | 92,471 | 85,966 | 0.92965 |
| Júlio de Castilhos | 56,352 | 11,195 | 0.19866 |
| Nova Palma | 16,197 | 2,837 | 0.17516 |
| Pedro Osório | 33,847 | 12,133 | 0.35847 |
| Porto Alegre | 5,908 | 1,837 | 0.31093 |
| Rio Grande | 1,77,292 | 15,344 | 0.08655 |
| Sant'Ana do Livramento | 5,31,504 | 3,36,185 | 0.63252 |
| Santa Maria | 6,37,390 | 1,13,981 | 0.17883 |
| Santa Rosa | 2,05,398 | 9,132 | 0.04446 |
| Santiago | 5,57,094 | 179.853 | 0.32284 |

[1] data derived from Headley et al., Br. J. Microbiol. 2020, 51: 1405–1432, and Rech et al. Pesq Vet Bras. 2005; 25: 97-105; [2] IBGE, 2024.

recent identification of OvGHV2 in asymptomatic wild boars from southern Brazil may be the key to explain these unusual outbreaks of SA-MCF in several regions of this country [10], considering the habits and the extensive presence of wild boars throughout this continental nation [35].

### *Adult sheep were the source of this outbreak*

An interesting finding was the demonstration of OvGHV2 in the blood of two asymptomatic adult sheep. Since these were the only sheep maintained at this farm it is rather likely that they served as sources of infection (as mentioned previously). However, the peak of shedding of OvGHV2 lasts for 36 hours, and is predominantly more elevated in adolescents (between 6 – 9 months of age) relative to adult sheep, with a single replication cycle in each animal [36]; the two sheep maintained at this farm were between 1.5 – 2 years of age. Nonetheless, OvGHV2 DNA was detected in several categories of sheep, including animals that were more than 2 years of age, while there was a statistical difference between the pregnant and post-partum ewes [37], and frequently, an entire sheep herd may be subclinically infected [4,9]; therefore, all sheep can serve as sources of infection to susceptible mammalian populations. These findings suggest that the two-adult sheep at this farm could have served as disseminators of this outbreak. Furthermore, it is not known if sheep rearing was done on another farm within proximity to the location where this outbreak occurred. Nevertheless, the epidemiological data identified in this outbreak is similar to that described in most geographical regions of Brazil [1] and worldwide [4], resulting in low morbidity but elevated lethality.

### *The fascinating epidemiology of SA-MCF in the State of Rio Grande do Sul*

The State of Rio Grande do Sul is a major player in cattle and sheep rearing and production in Brazil, since in 2022 it had the third principal population of sheep (15.59%; 3,353,607/21.514.274) and was home to the eighth largest number of cattle (5.09%; 11,932,838/234,352,649) reared in the country [22]. Epidemiological data have demonstrated that most of the published outbreaks of SA-MCF reported in animals from Brazil occurred in diverse geographical regions of RS [1,24] as compared to other Federative member States [1]. The first outbreak of SA-MCF in RS occurred within the Central West mesoregion in 1973 [38] and represented the third reported description of this disease in Brazil. Although the reason for the comparatively elevated number of SA-MCF outbreaks in RS is unknown, the type of sheep production traditionally practiced in that state may be the key.

Due to the large number of outbreaks of SA-MCF in RS, a seasonal occurrence with the predominance of outbreaks occurring during October to March was proposed [24], which would correspond from mid-spring to the end of summer in Brazil. Furthermore, lambing in RS occurs frequently during June to August [39], so by December to March (summer), sheep will be adolescents, having 6-9 months of age; period that is considered critical for the dissemination of OvGHV2 to susceptible animal populations [4,36]. Therefore, there seems to be a direct relationship between the period of lambing and the frequency of outbreaks of SA-MCF in RS.

Sheep rearing occurs in all mesoregions of the RS State, is predominantly extensive on pastures, with the principal objective being the production of meat and wool, and most sheep farmers adopt the integration rearing system where cattle is concomitantly maintained on the same pasture with sheep [40,41]. Additionally, this system of sheep rearing is predominant within the Pampa biome [26], where an elevated SCR was detected during this investigation. Consequently, all categories of sheep (young and adults) are reared simultaneously with cattle on the same pasture within the state of RS, which should have significantly increased the risk of SA-MCF outbreaks throughout all mesoregion of the state, since sheep are the reservoir hosts for OvGHV2 [2,6,9]. However, this study has statistically demonstrated that cattle reared within the municipalities of the Southeastern, Southwestern, Central Eastern, Northwestern, and Northwestern mesoregions of RS (i.e., most of the Pampa biome) have more elevated risk of developing outbreaks of SA-MCF due to the elevated SRC, as compared to cattle reared within geographical regions with SCR < 0.15. This statistical analysis then illustrates the possible reason for the occurrence of elevated numbers of SA-MCF outbreaks previously diagnosed within specific geographical locations of this state [1,24], suggesting that should sheep and cattle be reared on the same farm, at least within the RS state, the SCR must be less than 0.15 to reduce the possibility of an outbreak of SA-MCF. Consequently, the chances of an outbreak of SA-MCF occurring within the municipality of Selbach, Northwestern mesoregion of RS is comparatively reduced and may explain the reason for only one additional known outbreak of SA-MCF within this mesoregion [24]. These findings demonstrated that, in general terms, the SCR correlated well with the identification of previously reported outbreaks of SA-MCF within RS, suggesting that this index can be used as a possible predictive indicator of the chance of an SA-MCF occurring in locations where sheep and cattle are reared concomitantly. Therefore, the more elevated the SCR, the greater the chance of an SA-MCF outbreak occurring in a determined geographical region.

However, since this index only considers the number of sheep and cattle reared concomitantly within one specific area, other risk factors specific to each geographical location, but not considered in this study, could have also contributed towards the development of the SA-MCF outbreaks, particularly in regions with an SCR close to the cut-off limit. Consequently, several factors may affect the occurrence of SA-MCF in any given ruminant population and must be considered in conjunction with the SCR ratio. These factors include: a) the pathogenicity of the specific strains of virus circulating within each geographical; b) the number of viral particles shed by sheep with the potential to infect populations of ruminants [42]; c) the distinct husbandry and environmental conditions within a specific geographical region [42,43]; d) specific herd genetics that favor virus transmission [42]; e) misdiagnosis or underdiagnosis of SA-MCF due to confusion with similar clinical infectious diseases of cattle [e.g., infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD)], and the clinical manifestations of plant intoxication [27]; and f) the simultaneous occurrence of plant toxicosis and infections by OvGHV2 [44]. A special note must be given to plant intoxication in ruminants since this is a major contributor to cattle mortality within RS [45]; however, in most cases, the possible participation of simultaneous disease conditions is not investigated. Therefore, the SCR should be used in conjunction with other risk factors associated with the development of SA-MCF outbreaks in locations in RS where the calculated index is close to the cut-off point.

Even though there is no current commercial vaccination available for SA-MCF, the separation of sheep from susceptible animal populations and the non-concomitant rearing of sheep and cattle [27] are advocated control strategies to reduce infection by OvGHV2. Consequently, the system of sheep rearing and production practiced in the state of Rio Grande do Sul, ironically, favors the maintenance and propagation of OvGHV2 within asymptomatic sheep populations. Therefore, it is recommended that sheep producers, veterinarians, and government officials from the State of RS adopt prophylactic measures to mitigate the frequency of SA-MCF outbreaks.

### *Study limitations*

There were several setbacks in this study that would have provided excellent transmission information for the epidemiology of OvGHV2 in Brazil. The first was the pooling of samples which prevented the exact determination of infection in each sample evaluated as well as the two asymptomatic sheep. However, this strategy was necessary and understandable since the samples were submitted exclusively for diagnostic purposes. The second was the non-quantification of the number of copies of OvGHV2 DNA detected in each sample by qPCR. This would have been fundamental to evaluate the viral DNA load in the two carrier animals to establish infection and their actual capacity to disseminate the virus to the susceptible calf; this is because OvGHV2 infection in sheep requires the detection of elevated copies (at least $10^5$) of viral DNA [46]. Therefore, the simple detection of OvGHV2 DNA in sheep, as was done in a previous study by only conventional PCR [37], does not necessarily imply infection. Consequently, confirmation of OvGHV2 infection in the asymptomatic host requires the quantification of viral DNA by qPCR and/or the detection of intralesional tissue antigens or proteins of OvGHV2 by immunohistochemistry or *in situ* hybridization, respectively. Nevertheless, these limitations had no effect on the diagnosis of SA-MCF and the epidemiological data presented herein.

A limitation with the utilization of SCR is the number of undiagnosed cases of SA-MCF in any geographical area that could have been confused with infectious diseases of cattle with similar clinical manifestations (e.g., IBR and BVD) as well as the clinical manifestations observed in cattle due to the ingestion of specific toxic plants. Therefore, misdiagnosis will affect the number of clinical cases of SA-MCF and have effects on the SCR. Arguably the SCR will have to be molded to each specific geographical location since local farming practices may have some effect on the results.

## Conclusions

OvGHV2 DNA was identified by qPCR in multiple organs of a calf that developed clinical manifestations consistent with SA-MCF. Additionally, qPCR detected OvGHV2 in two asymptomatic sheep that were reared concomitantly with this calf. The SCR was proposed to estimate the chance of outbreaks of SA-MCF in cattle to occur and demonstrated that reported outbreaks of SA-MCF were predominant within geographical regions of Rio Grande do Sul, Southern Brazil, with an SCR $< 0.15$. Furthermore, the concomitant rearing of sheep and cattle coupled with the sheep rearing system traditionally practiced within the state of Rio Grande do Sul seem to favor the development of SA-MCF outbreaks within this geographical region of Brazil.

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## Data Availability

The datasets analysed during the current study are available in the IBGE (Instituto Brasileiro de Geografia e Estatística) reprisotory, https://www.ibge.gov.br/.

## Authors Contributions

Conceptualization, Headley, S.A.H.; formal analysis, map design, and statistical evaluations, F.D.C.M.; investigation, S.A.AH., F.D.C.M., and D.L.R.; data obtention: D.L.R.; data curation, S.A.H., and F.D.C.M.; writing—original draft preparation, S.A.H.; writing—review and editing, S.A.H., F.D.C.M, and D.L.R.; project supervision, administration, and funding acquisition S.A.H. All authors have read and agreed to the final version of the manuscript.

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## Conflict of interests

No conflict of interests is declared.

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