Prevalence and risk factors associated with Maedi-Visna infection in sheep in the State of Maranhão, Brazil

Prevalência e fatores de risco associados à infecção por Maedi-Visna em ovinos no Estado do Maranhão, Brasil

Prevalencia y factores de riesgo asociados a la infección por Maedi-Visna en ovinos de Estado de Maranhão, Brasil

Abstract

In order to determine Maedi-Visna virus (MVV) seroprevalence and risk factors associated with infection in sheep, 445 animals of both sexes and different ages were tested using the Agarose Gel Immunodiffusion technique (IDGA). The animals were divided into two groups: group 1 composed of exhibition animals (n=70) and group 2 composed of animals from properties from the north, east and central mesoregions of the state of Maranhão (n=375). The general prevalence of MVV infection was 2.02%; 1.42% in group 1 and 2.13% in group 2. In the north mesoregion the prevalence was 2.20%, while a total of 40% of the animals living in municipalities for MVV. It was observed that 1.15% of males and 2.23% of females were seropositive (p> 0.20). Regarding breeds, the Dorper (1.66%); Santa Inês (1.67%); White Dorper (33.33%) and Texel (4.34%) were the most affected. This was the only variable among all the risk factors studied that had a significant association in multivariate analysis (p <0.05). MVV infection is present regardless of the purpose of breeding, and the animals are exposed to the same risk of infection. This demonstrates the need to implement public policies for the prevention, control, and eradication of this disease.

Keywords: Lentivirus; Ruminant; Epidemiology.
os sexos, de diferentes razas e idades, sendo 70 do grupo 1 (animais de exposição), e 375 do grupo 2 (animais de propriedades das mesorregiões Centro, Leste e Norte maranhenses). Constatou-se uma prevalência geral da infecção pelo MVV de 2,02%, sendo 1,42% no grupo 1 e 2,13% no grupo 2. A mesorregião Norte apresentou prevalência de 2,20% sendo que, dos municípios da mesma, 40% dos animais estavam positivos para MVV. Observou-se que 1,15% dos machos e 2,23% das fêmeas foram soropositivos (p<0,20). Quanto às raças observou-se 1,66% Dorper; 1,67% Santa Inês; 33,33% White Dorper e, 4,34% para os da raça Texel, tendo sido a única variável entre todos os fatores de risco pesquisados, com associação significativa na análise multivariada (p<0,05). A infecção por MVV está presente independente da finalidade de criação, estando estes, expostos ao mesmo risco de infecção pelo MVV. Alerta-se para a necessidade de implantação de políticas públicas de prevenção, controle e erradicação dessa enfermidade.

**Palavras-chave:** Lentivirus; Ruminante; Epidemiologia.

### Resumen

Con el objetivo de determinar la seroprevalencia del virus Maedi-Visna (MVV) y los factores de riesgo asociados a la infección en ovinos, se investigó utilizando la técnica de Inmunodifusión en Gel de Agarosa (IDGA) 445 animales de ambos sexos, de diferentes razas y edades, siendo 70 del grupo 1 (animales expuestos) y 375 del grupo 2 (animales de propiedades de las mesorregiones Central, Este y Norte de Maranhão). Se constató una prevalencia general de la infección por MVV del 2,02%, siendo 1,42% en el grupo 1 y 2,13% en el grupo 2. La mesorregión Norte tuvo una prevalencia del 2,20%, siendo que, de los municipios de la misma, el 40% de los animales fueron positivos para MVV. Se observó que el 1,15% de los machos y el 2,23% de las hembras fueron soropositivos (p<0,20). En cuanto a las razas, se observó un 1,66% Dorper; 1,67% Santa Inês; 33,33% White Dorper y, 4,34% para los de la raza Texel, habiendo sido la única variable entre todos los factores de riesgo encuestados, con asociación significativa en el análisis multivariado (p<0,05). La infección por MVV está presente independientemente de la finalidad creación, estando éstos, expuestos al mismo riesgo de infección por MVV. Se advierte de la necesidad de implementar políticas públicas para la prevención, control y erradicación de esta enfermedad.

**Palabras clave:** Lentivirus; Ruminante; Epidemiologia.

### 1. Introducción

Maedi-Visna (MV) es una enfermedad crónica y progresiva multisistemática que principalmente afecta la oveja (Aratújo et al., 2004; Lombardi et al., 2009). Se debe a un virus de RNA perteneciente al género Lentivirus, familia Retroviridae, subfamilia Lentivirinae (Costa et al., 2007). La infección se caracteriza por el desarrollo de lesiones inflamatorias crónicas en diferentes órganos, principalmente en el pulmón, glándulas mamarias y el sistema nervioso central (CNS). En el CNS, un patrón histiocítico o linfocítico inflamatorio es predominantemente descrito (Gayo et al., 2018). Los animales con un patrón histiocítico son fácilmente reconocibles por el test ELISA, mientras que las vacas con un patrón linfocítico pueden pasar inadvertidas como seronegativas y probablemente sean un remanente del fuente de infección (Gayo et al., 2017).

La enfermedad se propaga de manera fácil y las condiciones de manejo pueden favorecer su propagación entre animales. Sin embargo, ya que los principales métodos de propagación son el contacto directo con las secreciones ricas en células del sistema mononuclear-fagocítico, como el sangre, el leche o el colostrum (Callado et al., 2001; Blacklaws et al., 2004; Silva & Lima, 2007). Adicionalmente, la propagación puede ocurrir principalmente a través de exudados respiratorios y secreciones, más a menudo facilitada entre animales mantenidos en encierros (Callado et al., 2001; Cortez-Romero et al., 2013).

Entre los tests serológicos disponibles, el Agarose Gel Immunodiffusion (AGID) está recomendado por la Organización Mundial para la Salud Animal (OIE) y es ampliamente utilizado para el diagnóstico de pequeños rumiantes en la mayoría de los países del mundo (OIE, 2012).

Los aglomerados del ganado representan un importante vínculo en la propagación de enfermedades infecciosas (Alves et al., 2017). En ausencia de vacunas eficaces, es de gran importancia entender el epidemiología de esta enfermedad para limitar su propagación. Muchas variables influyen en los costos esperados y beneficios de los programas de control y deben ser evaluados en orden de implementar medidas que conduzcan al control de esta enfermedad en ganados de vacuno (Mingujón et al., 2015).

El daño económico causado por esta enfermedad es considerable, ya que puede resultar en un considerables pérdidas de peso en animales, fallas reproductivas, el necesidad de reemplazo de reproductoras y pérdidas de ganado; así como la limitación de comercio internacional debido a los obstáculos sanitarios que puede causar. La falta de información sobre Maedi-Visna Virus (MVV), su distribución y...
dispersion has contributed to a lack of effective control measures. The aim of the present study was to determine the prevalence and identify risk factors associated with Maedi-Visna Virus in sheep from the State of Maranhão.

2. Methodology

The study was approved by the Ethics Committee in Animal Experimentation of the State University of Maranhão (CEAA/UEMA – 012/2013).

The animals selected for the present study were divided into two groups. Group 1 was composed of exhibition animals which participated in the 57th Agricultural Exhibition of the state of Maranhão (EXPOEMA), and which belonged to exhibitors from the states of Pernambuco, Piauí and Maranhão. Group 2 was composed of animals raised in properties in the municipal regions of Itapecuru-Mirim, Paço do Lumiar, Raposa, Santa Rita, São José de Ribamar, São Luís (north mesoregion of state of Maranhão), São Luís Gonzaga (central mesoregion of state of Maranhão) and Timon (east mesoregion of state of Maranhão).

In total, 445 purebred sheep were included in the survey. Of these, 87 were male and 358 were female, aged from two months to eight years, of the Dorper, Santa Inês, White Dorper and Texel breeds.

The number of animals in Group 1 was determined by the non-probabilistic sampling technique (Thrusfield, 2004). The owners were selected by voluntary participation after signing a free and informed consent form. A total of 70 animals (35 female and 35 male) of the Dorper, Santa Inês and White Dorper breeds were selected.

Data provided by the Brazilian Association of Sheep Breeders (ARCO) from the year 2012 was used to determine the amount of animals in Group 2. The number of samples was determined by simple randomization, according to Triola (1999) and Callegari-Jacques (2003), based on an expected prevalence of 50%, a sample error of 5% and a confidence level of 95%, resulting in a minimum of 361 animals. Property selection was carried out by voluntary participation, irrespective of the number of animals in each property. Samples of all the defined-breed sheep present on the selected properties were collected, totaling 375 animals (52 males and 323 females) of the Dorper, Santa Inês, White Dorper and Texel breeds.

A total of 10 mL of blood was collected from the external jugular vein. The samples were conditioned and sent to the Veterinary Clinical Pathology Laboratory of the State University of Maranhão (UEMA). The serum was placed in microtubes and stored at -20°C until the serological tests were performed.

The detection of Maedi-Visna virus antibodies was performed using the agar gel immunodiffusion technique (AGID), with a commercial kit (Biovetech®, Recife, Pernambuco, Brazil) and following the manufacturer's recommendations. Two readings were performed, one after 24h and one after 48h, by three independent observers. The result was considered that taken after the second reading. Samples that tested positive were repeated three times to confirm the result.

Properties were considered positive for the presence of the Maedi-Visna Virus when at least one reagent animal was found. First, the prevalence of seropositive animals was estimated and then a descriptive statistical analysis of the groups by sex, breed and age was performed. The frequencies in the studied strata were calculated based on the serological results by simple percentage.

The variables that were related in the epidemiological survey allowed the study of risk factors. A database was generated, with the animal considered to be an epidemiological unit, to identify which of the studied factors were associated with the Maedi-Visna virus. The logistic regression model was used (Hosmer & Lemeshow, 1989).

Data were analyzed in the STATA 9.0 program. Univariate analysis of each independent variable was performed by selecting those with a p-value <0.20. Subsequently, the combined association of the factors studied with respect to the occurrence of Maedi-Visna was analyzed using the multivariate logistic regression technique, with only the variables that presented a p-value <0.05 remaining in the model. Prevalence ratios (PR) and 95% confidence intervals were estimated. The level of
significance adopted was 5%. The comparison of the frequencies between groups was performed by the chi-squared test and logistic regression analysis was used to identify risk factors with a significant association with seropositivity.

3. Results

Of the total number of animals evaluated, 19.55% (87/445) were male and 80.45% (358/445) were female. Seroprevalence was 1.15% (1/87) in males and 2.23% (8/358) in females. Among the positive animals, there was a predominance of females, with 88.9% (8/9), while only 11.10% (1/9) of positive cases were male. No risk association was found between sex and the occurrence of the disease (p> 0.20) (Table 1).

Table 1. Risk factors associated with the occurrence of the Maedi-Visna Virus (MVV) in non-defined breed sheep in the State of Maranhão (2014).

| Variable | Reagents/exposed | %   | Odds ratio | P value |
|----------|------------------|-----|------------|---------|
| Sex      |                  |     |            |         |
| Female   | 8/358            | 2.23|            | 0.51    |
| Male     | 1/87             | 1.15| 0.51       | 0.53    |
| Age      |                  |     |            |         |
| <12 months | 1/76          | 1.31|            |         |
| 12-16 months | 1/32        | 3.12| 2.42       | 0.53    |
| 17-22 months | 0/15        | 0   |            |         |
| 23-36 months | 1/119        | 0.84| 0.63       | 0.75    |
| 37-48 months | 1/51         | 1.96| 1.5        | 0.78    |
| > 48 months | 5/152         | 3.28| 2.55       | 0.39    |

**p<0.20 - statistically significant. Source: Authors.

In terms of age, 17.07% (76) of the animals were up to 12 months old; 7.20% (32) were aged between 12 and 16 months; 3.37% (15) between 17 and 22 months; 26.75% (119) between 23-36 months; 11.46% (51) between 37 and 48 months and the largest portion of the sample, 34.15% (152), was aged over 48 months. This older age group also contained the largest number of reactive animals, with 3.28% (5/9). Univariate logistic regression analysis did not reveal a statistically significant association (p> 0.20) between age group and the occurrence of MVV (Table 1).

The general prevalence of the Maedi-Visna Virus (MVV) without clinical signs characteristic of the disease was 2.02% (9/445, 95% CI = [0.93 - 3.80]). The breed (OR=29.5, CI 95% = [1.83 – 474.41]), amount of animals in the property (OR=10.1, CI 95% = [1.05 – 96.61]), type of updating of knowledge of breeders (OR = 8.6, 95% CI = [0.46 - 159.83]) and feeding of lambs (OR = 10.63, 95% CI = [1.09 - 103.23]) had a statistically significant relationship (p <0.20) with the MVV infection of the animals in the univariate logistic regression analysis of risk factors (Table 2).
Table 2. Risk factors for Maedi-Visna Virus in defined breed sheep in the State of Maranhão (2014).

| Breed          | Reagents/exposed | %   | Odds ratio | P value |
|----------------|------------------|-----|------------|---------|
| Dorper         | 2/120            | 1.66|            |         |
| Santa Inês     | 5/299            | 1.67| 1.00       | 0.99    |
| White Dorper   | 1/3              | 33.33| 29.5       | 0.02**  |
| Texel          | 1/23             | 4.34| 2.68       | 0.43    |

**p<0.05 - statistically significant. Source: Authors.

Variables with a p value less than 0.20 (p <0.20) were included in the multivariate logistic regression model; the "breed" variable was the only risk factor with a statistically significant association (p <0.05). White Dorper animals were 44 times more likely to be infected with MVV than the other breeds tested. Reagent animals were observed in all the evaluated breeds; 1.66% (2/120) for the Dorper breed, 1.67% (5/299) for Santa Inês, 33.33% (1/3) for White Dorper and 4.34% (1/23) for Texel (Table 2).

Of all the positive animals, 2.20% (9/409) were from the north mesoregion of the state of Maranhão. Of all the municipal regions, 40% (4/10) had at least one property with a seropositive animal.

A prevalence of 1.42% (1/70) in group 1 and 2.13% (8/375) in group 2 was observed (Table 3). In terms of the overall prevalence of the disease, Group 1 had 0.22% of seropositive animals and group 2 had 1.80% of seropositive animals. Statistical analysis, however, revealed that the frequencies of MVV did not differ between the groups.

Table 3. Prevalence of Maedi-Visna, per group of sheep tested by agarose gel Immunodiffusion (AGID) in the State of Maranhão (2014).

| Group | Reagents/tested animals | Reagents (%)\(^1\) | Reagents (%)\(^2\) | \(\chi^2\) (*) |
|-------|-------------------------|--------------------|--------------------|---------------|
| 1*    | 1/70                    | 1.42               | 0.22               | 0.15          |
| 2*    | 8/375                   | 2.13               | 1.80               |               |

\(^1\) Percentage of reactive sheep compared to the total tested in each stratum.
\(^2\) Percentage of reagents sheep compared to all animals tested in the study.
\(*\) \(\chi^2\) values > than 3.84 - significant association with p value.

Source: Authors.

4. Discussion

The general prevalence of the Maedi-Visna (MV) found in this study is considered low by Reina et al. (2009) (1 to 9%), and is similar to that observed in other states where a semi-extensive breeding system also predominates. This may be related to the recent introduction of the virus in the region and to the fact that this rate varies greatly in purebred animals, according to Batista et al. (2004).

Low prevalences were also found in the state of Pernambuco (1.1%) by Costa et al. (2007), Bahia (0.5% and 0.34%) by Souza et al. (2007) and Martinez et al. (2011) and in Tocantins (0.9%), by Moura Sobrinho et al. (2008). In studies conducted by Guilherme et al. (2017), none of the animals tested were positive for MVV. Gregory et al. (2013) evaluated 226 sheep samples from the state of São Paulo, in the microregions of Botucatu, Campinas, Piedade and São Paulo, and found that none of the samples were seroreagent.
The analysis of risk factors for the occurrence of MV revealed a result that contradicted the findings of Moura Sobrinho et al. (2008) and Lombardi et al. (2009). However, results presented in this work corroborate with data from Sobrinho et al. (2010) findings of the present study, as they also found a significant association between breed and the occurrence of Lentiviriosis in small ruminants in the state of Tocantins. Callado et al. (2001) stated that breed susceptibility could not be identified, as the studies are difficult to interpret due to various factors related to handling.

The null prevalence found in the other states surveyed and in the central and east mesoregions can be explained by the reduced number of animals tested. However, the presence of reactive sheep in the north mesoregion may be due to the fact that this region had the highest concentration of sheep breeders in the study area, as well as the greatest number of cities, properties and animals sampled. Teixeira et al. (2016) observed an overall prevalence of MVV of 0.7%, and prevalences of 0.5%, 0.7% and 1% in the central, eastern and northern mesoregions of the state of Maranhão, respectively.

Studies carried out by Moura Sobrinho et al. (2008) observed that there was a numerically greater number of positive cases among animals of the Santa Inês breed (6/511) than among non-defined breed animals (2/324). However, no significant association was found between breed and MVV occurrence. In the results obtained by Teixeira et al. (2016) there was no association between seropositivity and the breed affected.

The lack of risk association between sex and the occurrence of the disease (p> 0.20) corroborates the results of Lombardi et al. (2009) and Sobrinho et al. (2010). The same was observed between age group and the occurrence of MVV, similar to the findings of Moura Sobrinho et al. (2008). In the study by Alves et al. (2017), all the sheep male were negative and 6.72% (8/119) sheep female were seropositive. Teixeira et al. (2016) observed that 0.5% (1/207) of seropositive sheep were male and 0.8% (10/1288) were female.

The claim that MVV infection can affect animals of any age was verified in studies by Rowe & East (1997), where it was observed that animals aged less than one year and older than 48 months were seropositive, and that a higher percentage of infection was found in the latter stratum (59 - 3.28%). According to Snowder et al. (1990) and Cutlip et al. (1992), older animals are exposed to the infectious agent for a longer period while seroprevalence tends to be higher in this age group, as it is a chronic disease with a long, slow course, allowing the confirmation of seroconversion. Teixeira et al. (2016) also found that there was no significant difference in this variable.

There were no animals with clinical signs of MVV, probably due to the long incubation period of the virus, a finding similar to the observations of Araújo et al. (2004), Souza et al. (2007) and Lombardi et al. (2009). This result reinforces the hypothesis that the infections are recent.

5. Conclusion

Maedi-Visna Virus infection is present in non-defined breed sheep in the state of Maranhão, and all sheep are exposed to the same risk of infection irrespective of the purpose of breeding. The occurrence of Maedi-Visna indicates the need to implement preventive and control measures, especially as these animals are used as herd enhancers, and have the capacity to spread the disease quickly and across a large geographic area.

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Ethics Committee in Animal Experimentation

The study was approved by the Ethics Committee in Animal Experimentation of the State University of Maranhão (CEEA/UEMA – 012/2013).

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