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# *Detection of antibodies to Visna/Maedi in sheep from Recôncavo Baiano*

## *Ocorrência de anticorpos contra Visna/Maedi em ovinos na região do Recôncavo Baiano*

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**Abstract:** The Visna/Maedi virus is a *Lentivirus* that causes a multisystem, progressive, slow-developing disease that can affect sheep and goats. After long periods of subclinical infection, the disease can slowly progress to cause the degeneration of various organs, cachexia and death. The diagnosis is made by the observation of clinical signs and confirmed by serological tests and isolation and/or identification of the virus. There is no treatment for the disease. The control is based on segregated breeding, handling and slaughtering of the positives. As Visna/Maedi is on the list of the World Organization for Animal Health (OIE), reporting of the disease is obligatory and it has been found in some States in Brazil, causing negative economic impacts with its spread. Given the limited seroepidemiological data available in the Brazilian State of Bahia, this study aimed to carry out a seroepidemiological survey to investigate the occurrence of anti-Visna/Maedi virus antibodies in sheep from Recôncavo Baiano. Two hundred serum samples from sheep were subjected to the agar gel immunodiffusion test (AGID). None of the serum samples were found to be positive. To confirm the absence of Visna/Maedi in sheep herds in Bahia, there is a need for further studies with significant sampling including a more sensitive assay.

**Resumo:** O Visna/Maedi virus (MVV) é um *Lentivirus* que causa uma enfermidade multissistêmica, progressiva e de curso lento, que pode acometer ovinos e caprinos. Após longos períodos de infecção assintomática, pode progredir lentamente à degeneração de vários órgãos, caquexia e morte. O diagnóstico se faz através da observação de sinais clínicos e a confirmação mediante testes sorológicos, isolamento e/ou identificação do vírus. Não há tratamento, até o momento. O controle é baseado na criação segregada, manejo e sacrifício dos positivos. Por fazer parte da lista da Organização Mundial de Saúde Animal (OIE), ser uma doença de notificação compulsória, ter sido verificada em alguns estados do Brasil, podendo causar impactos econômicos negativos com a sua disseminação, e haver escassos dados soroepidemiológicos no Estado da Bahia, esta pesquisa teve por objetivo realizar inquérito sorológico para investigar a ocorrência de anticorpos para Visna/Maedi em ovinos, na região do Recôncavo Baiano. Duzentas amostras de soros ovinos foram submetidas ao teste de imunodifusão em gel de agarose (Idag). Nenhum dos soros pesquisados foi reagente. Para que se possa realmente confirmar a ausência de Visna/Maedi nos rebanhos de ovinos na Bahia, há necessidade de outros estudos com amostragem significativa e a inclusão de testes mais sensíveis.

## 1 Introduction

*Small Ruminant Lentivirus* (SRLV) is a generic term used to describe the etiologic agents of caprine arthritis encephalitis (CAE) and Visna/Maedi (Visna). These viruses belong to the *Retroviridae* family and affect goats and sheep, are widespread and phenotypic, biological and antigenically related (PASICK, 1998). The Visna is a disease of sheep and it is chronic, progressive and multisystemic, which mainly affects lungs, mammary glands, brain and more rarely, joints (PUGH, 2002), causing economic losses due to the decrease in productive life and reproductive efficiency, delay in growth, increased mortality of offspring, and indirect losses resulting from the devaluation of the herds, early replacement of diseased animals, costs control measures and possible trade barriers, which may limit or prevent the international trade (SILVA et al., 2005).

The SRLV transmission is mainly via ingestion of colostrum and milk of infected females or through the respiratory contact, more common in intensive system or via contaminated semen though with lesser risk (BLACKLAWS et al., 2004). According to Leginagoikoa et al (2006), there is evidence that horizontal transmission is very low, despite the prolonged direct contact between infected and uninfected sheep, highlighting the need to investigate the dynamics of virus excretion and transmission among animals to obtain grants and to establish control in endemic populations of Visna. Furthermore, phylogenetic studies have demonstrated the SRLV transmission in sheep to goats and vice versa (LEROUX et al., 1997; SHAH et al., 2004; GJERSET et al., 2009; GLARIA et al., 2009).

Diagnosis of SRLV can be made through observation of clinical signs, but due to the SRLV characteristics of persistent infection and no symptoms in most infected animals, the confirmation is made through serologic tests, or isolation or identification of the virus. The most way extensively used to diagnose SLRV is to perform serology by the agar gel immunodiffusion (AGID), due to be recommended by the World Organisation for Animal Health (OIE, 2004), to present low cost and high specificity and practicality (PUGH, 2002). However, the test has low sensitivity in the early phase of infection (LEROUX et al., 1997; FLEET et al., 2005; ELTAHIR et al., 2006).

So far, there is no treatment for SRLV and control is based on the reduction of infection with the implementation of appropriate management with segregated breeding and slaughter of the positives (SMITH, 1994; REINA et al., 2009).

Several authors reported the presence of antibodies to Visna in Brazil and identified in Rio Grande do Sul (MOOJEN et al., 1995; RAVAZZOLO et al., 1995; RAVAZZOLO et al., 2001); in São Paulo (FERNANDES et al., 2003); in Rio Grande do Norte (SILVA et al., 2003); in Ceará (ALMEIDA et al., 2003; ARAÚJO et al., 2004); in Pernambuco (OLIVEIRA et al., 2006; COSTA et al., 2007). However, the first reports in the State of Bahia occurred recently in Juazeiro by Souza et al. (2007) and Martinez et al. (2008).

Thus, considering the negative economic impact caused by spread of Visna, and the disease is included in list of OIE as a disease of compulsory notification, and has already been verified in some States of Brazil, and the lack of seroepidemiologic data in Bahia, this research aimed to carry out a seroepidemiological survey to investigate the occurrence of antibodies to *Visna/Maedi virus* (Visna) in sheep from Recôncavo Baiano Region.

## 2 Material and Methods

Two hundred sheep sera samples from various breeds were examined, convenience sampling, included 43 males and 157 females, aged five months to eight years. There was no criterion of probability applied to establish the number of animals in this study. In this way, all analyzed samples were harvested from nine properties as belonging to eight municipalities of the Recôncavo Baiano Region: Santo Amaro, Castro Alves, Jaguaripe, Jequiriça, Muritiba, Governador Mangabeira, Mutuípe and Laje. These municipalities were contemplated according to the acceptance of farm owners and the ease of access; therefore, there was no criterion for selection of sampling. The collection of samples was carried out from October 2006 until January 2007. In some herds, animals with respiratory symptoms were identified coexisting with asymptomatic animals.

Blood samples were harvested in sterile glass tubes without anticoagulant by puncture of the external jugular vein, and a total volume of 10 mL per animal were obtained. The tubes were placed in vertical position to the coagulation, and identified with numbers and properties names, and then packed in styrofoam boxes filled with dry ice and sent to the Laboratories of Bacteriosis and Virosis of the Veterinary Medicine School/ UFBA. After clot

retraction, the blood samples were centrifuged at 2000 g for 10 minutes, and the sera were placed in 1.5 mL polypropylene microtubes (Eppendorf) at -20 °C until the performance of serological tests (ALMEIDA et al., 2003; OLIVEIRA et al., 2006 ). At the time of collection of the biological material, a clinical evaluation was carried out in the animals to detect suggestive alterations of Visna infection.

Serology for detection of antibodies against Visna was carried out by the AGID (OIE. 2004), using a kit produced by the Federal Rural University of Pernambuco, consisting of antigen produced in cell cultures infected with Visna (protein - p28), and positive serum harvested from naturally infected animal. The AGID test was performed according to the manufacturer's recommendations and followed the OIE recommended pattern. The reading of the results was obtained in 24, 48 and 72 h of incubation in a moist chamber, and the definitive reading was in the third period.

## 3 Results and Discussion

According to the clinical examination, five herds (5 / 9) presented animals malnourished, with respiratory alterations or mastitis, indicating a possible presence of Visna. However, serologic evidences of viral infection were not observed in two hundred ovine serum samples submitted to AGID test for detection of antibodies against Visna in this study (Figure 1).


**Figure 1.** Negative results in the definitive reading of Agid test after 72 hours of incubation in a moist chamber. PS – positive serum; AG – antigen; SS – serum sample.

A characteristic of SRLV infection is a high prevalence of seropositive animals, apparently healthy, in other words, most of infected small ruminants do not develop clinical signs (PUGH, 2002). In this study, five of nine herds were presenting animals with clinical signs of Visna. However, all the sampled animals were negative by AGID.

Serologic studies for detection of Visna were carried out in the State of São Paulo by Fernandes et al. (2003) that obtained 2.8% of sheep with Visna infection. In the northeast of Brazil, some authors performed serologic studies and the found results were 21.3% and 31.67% of animals seropositive for Visna infection in the States of Rio Grande do Norte (SILVA et al., 2003) and Ceará (ALMEIDA et al., 2003) respectively. However, Oliveira et al. (2006) and Costa et al. (2007) report data in their studies of 5.2% and 1.07% of Visna animal reagents for the state of Pernambuco. Similarly as Araújo et al. (2004) that found 4.93% seropositive sheep for Visna in the State of Ceará. One of the determinants that may explain the different results is the existence of epidemiological or geographical barriers between the areas mentioned in the studies described above and those obtained in the present research. However in the Bahia State, Souza et al. (2007) identified only 0.5% of reagents animals (1 / 200) of two hundred samples of sheep sera from Juazeiro / Bahia, demonstrating the possible presence of *Lentivirus* in the State of Bahia. And Martinez (2008) also in Juazeiro/BA identified 0.34% of sampled sheep positive by AGID.

Despite these values quantitatively low, the mentionated results above differ greatly from those in this study, since none of all the samples analyzed was reagent for the AGID. Some possible reasons for this result are the lower sensitivity of the Agid assay used, and late seroconversion, and low number of tested animals. Thus, the absence of seropositive animals for Visna in this study is not evidence that this region of the State is free of Visna infection. Besides, there is evidence that cross-reactions are possible for SRLV, indicating a possible presence of Visna in goat herds and the virus spread. Then, further studies are necessary with significant sampling of sheep and goat population, as well as adoption of prevention and control measures to avoid the possible spread of the disease. In this regard, owners of sheep with genetic pattern high and economic value are very important to control the Visna, because the losses would increase with the Visna

spread in the Bahia State.

Besides, the difference among the results of this discussion may be justified by a possible modification of the protocol for AGID, as soon as the sensitivity of the method depends on the type of antigen used. According to Adams and Gorham (1986), the antigen produced with glycoprotein 135 (Gp135) has greater sensitivity to the AGID test than the p28 antigen used in this study. Another likely explanation may be due to slow production of antibodies, which can lead to late seroconversion of these animals that may occur months or years after infection; or the animals are at an early stage of infection that the production of antibodies to Visna is absent or low and can not be detected by the assay. This fact raises the frequency of false-negative results for Visna (LEROUX et al., 1997; FLEET et al., 2005; ELTAHIR et al., 2006), being a major problem in animals trade, because may favor the spread of the disease unnoticed inter-herds.

Furthermore, the AGID test and the enzyme-linked immunosorbent assay (Elisa), both are prescribed tests for international trade by the World Organisation for Animal Health (OIE, 2004). However the AGID is reported to be more specific, reproducible and simple to perform, but requires training and experience in reading the results (DE ANDRES et al., 2005). The Elisa is sensitive, cheaper and stages of the process can be automated (SAMAN et al., 1999), therefore making it useful for screening large numbers of sera. Thus, none of the two tests are perfects; the optimal choice of test depends on the purpose of testing, whether the objective is surveillance of a population or confirmation of suspected cases. Thereby, Toft et al. (2007) combined the AGID and commercially available indirect Elisas tests in a serial or parallel reading to improve the performance of diagnostic tests and found no significant improvement in the specificity, but the sensitivity was reduced significantly.

It is important to emphasize the hypothesis on the presence or absence of Visna in herds of the Recôncavo Baiano, because despite the absence of seropositive sheep in this research, recent phylogenetic studies have demonstrated the transmission of SRLV of sheep to goats and vice versa (LEROUX et al., 1997; SHAH et al., 2004; GJERSET et al., 2009; GLARIA et al., 2009). Based on these studies is not daring to speculate that the MVV can be present in small ruminants herds in the State of Bahia.

## 4 Conclusions

Therefore, one concludes that the animals sampled had no evidence of antibodies to Visna IDAG detectable by using viral p28 antigen at the collection period. Nevertheless, to confirm the absence of *Visna/Maedi* in sheep herds in Bahia, there is a need for further studies with significant sampling including a more sensitive assay.

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