Bovine Leukemia Virus in Bovine Aborted Fetuses

Kelly Cristina Santos Montanari1,2, Marcia Mayumi Fusuma1,3, Alessandra Maria Dias Lacerda2,4, Líria Hiromi Okuda2, Edviges Maristela Pituco3, Aline Feola de Carvalho4, Vanessa Castro5, Rosa Maria Piatti6, Eliana Scarcelli Pinheiro7, Ricardo Harakava8 and Claudia Del Fava8*

1Bolsista Mestrado CAPES, Programa de Pós-Graduação em Sanidade, Segurança Alimentar e Ambiental no Agronegócio, Instituto Biológico, São Paulo, SP, Brazil; 2Laboratório de Anatomia Patológica, Centro de Pesquisa de Sanidade Animal, Instituto Biológico, Sao Paulo-SP Brazil; 3Laboratório de Viroses de Bovídeos, Centro de Pesquisa de Sanidade Animal, Instituto Biológica, Sao Paulo-SP, Brazil; 4Bolsista Capacitação Técnica III FAPESP, Instituto Biológico, São Paulo, SP, Brazil; 5Laboratório de Doenças Bacterianas da Reprodução, Centro de Pesquisa de Sanidade Animal, Instituto Biológico, São Paulo-SP, Brazil; 6Laboratório de Bioquímica Fitopatológica, Centro de Pesquisa de Sanidade Vegetal, Instituto Biológico, São Paulo, SP, Brazil

ABSTRACT

We investigated the infection of Bovine Leukemia Virus (BLV) related to other pathogens (Neospora caninum, Bovine Herpesvirus-1 (BoHV-1), Bovine Viral Diarrhea Virus (BVDV), and pathogenic bacterial in 80 bovine aborted fetuses. The materials comprised whole fetuses, fetal organs, and placenta. The BLV was diagnosed by nested-PCR (env gp51 BLV gene), the identification of viral genotypes by sequencing, and the phylogenetic analysis by neighbor-joining and maximum composite likelihood methods. The other pathogens and diagnoses were, respectively: Neospora caninum (nested-PCR), BoHV-1 (nested-PCR), BVDV (PCR), Brucella spp. (isolation and identification), Leptospira spp. (PCR), aerobic bacteria (Enterobacteriaceae, Gram positive cocci, Trueperella (Arcanobacterium) pyogenes) and micro-aerophilic (Campylobacter spp., Histophilus somni, and Listeria monocytogenes) by isolation and identification. BLV fetal antibodies were identified by ELISA kit. Thirteen (16.25%) fetuses were positive by BLV nested-PCR. Phylogenetic analysis revealed BLV genotypes 1, 5, and 6, which are frequently found in cattle in Brazil, Argentina, and Uruguay. No fetuses were positive for BLV antibodies by ELISA. A single case of coinfection with BLV was found for each of the pathogens Trueperella (Arcanobacterium) pyogenes, Klebsiella spp., and Streptococcus spp. were isolated as a pure or representing the preponderance of bacteria in a pooled culture. In the 67 BLV-negative fetuses, pathogens identified were single cases of Trueperella (Arcanobacterium) pyogenes, Staphylococcus aureus, and Brucella abortus; 2 of Escherichia coli; 3 of bovine viral diarrhea virus; and 4 of Neospora caninum. No pathogens were found in 55 fetuses. The low number of BLV positive samples infected or no by other pathogens didn’t allow performing statistical analysis, in order to understand if there were significative differences among not infected and infected BLV fetuses. Because BLV is an immunosuppressive agent and predisposes the cow to other pathogens, its connection with Leukemia or abortions need additional studies with bigger sampling, for elucidating pathogenesis in the pregnant cow and in the fetus. The rates of BLV transplacental transmission show the necessity of prophylactic measures in Brazilian cattle herds, in order to avoid infection in utero.

Keywords: Abortion; Bovine leukemia virus; Cattle; Fetuses; Filogeny; Histopathology; Nested PCR

INTRODUCTION

Abortion and neonatal mortality are frequent causes of economic loss in bovine production [1]. Infectious agents such as viruses, bacteria, and parasites can be involved [2-4]. It was reported that of 2,544 cases of bovine abortion, pathogens were found in only 30.2% [5]. In the majority, it is not possible to define the etiology, due to autolysis, multiple potential causes, and insufficient sampling of fetal tissues, placenta, and maternal and fetal serum [6]. Worldwide fetal death in cattle is estimated to be 5% [7], but abortion outbreaks, extended calving interval, loss of milk production, and lower pregnancy rates have been cited [8].

In Brazil were reported several infectious agents causing bovine abortion, due to etiologies of 2,544 cases of bovine abortion, pathogens were found in only 30.2% [5]. In the majority, it is not possible to define the etiology, due to autolysis, multiple potential causes, and insufficient sampling of fetal tissues, placenta, and maternal and fetal serum [6]. Worldwide fetal death in cattle is estimated to be 5% [7], but abortion outbreaks, extended calving interval, loss of milk production, and lower pregnancy rates have been cited [8]. In Brazil were reported several infectious agents causing bovine abortion; Bovine leukemia virus; Cattle; Fetuses; Filogeny; Histopathology; Nested PCR

Correspondence to: Claudia Del Fava, Laboratório de Anatomia Patológica, Centro de Pesquisa de Sanidade Animal, Instituto Biológico, Av. Conselheiro Rodrigues Alves, 1252, São Paulo-SP, Brazil, CEP: 04014-002, Tel: 5511 5087-1710; E-mail: delfava@biologico.sp.gov.br

Received: December 31, 2018, Accepted: January 21, 2019, Published: January 30, 2019


Copyright: © 2019 Montanari KCS, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Montanari KCS, et al.

abortion [9-12], but in 50% to 70% of cases direct diagnosis was not possible. Mononuclear inflammatory infiltrate has been observed in high rates in aborted fetal tissue, suggesting infectious causes [1,13]. Thus the improvement in sensitivity and specificity of laboratory techniques to investigate prevalent pathogens as well as other infectious agents not currently considered in a program of sanitary surveillance for abortion is important [2].

Bovine Leukemia Virus (BLV), a delta retrovirus in the family Retroviridae, is the causative agent of bovine leukemia [14], a chronic disease in which seropositive animals are carriers [15]. Thirty to 50% can present persistent lymphocytosis, and, of these, 2% to 5% may develop lymphosarcoma [15,16].

This disease reaches high rates of seroreactivity in intensively managed dairy cattle, and has been reported in all regions of Brazil [17]. There is no specific legislation for BLV control in the country, although it is recommended by the World Organization for Animal Health that countries importing live animals, semen, and embryos should certify them as BLV-free [18]. The high prevalence and wide distribution of BLV in Brazil suggests that the virus should be investigated as a potential pathogen involved in bovine abortion. A primary characteristic of BLV is mononuclear inflammatory infiltrate in multiple organs of immature and adult cattle [15,19] and this type of cell has been observed in aborted bovine fetuses without conclusive differential diagnosis, which may indicate an infectious etiology [19,13].

The intrauterine (vertical) transmission of BLV occurs in the first semester of gestation in up to 5% of seropositive pregnant bovines, mainly in those with high virus concentration and low antibody titers [20]. Serology of newborn calves before they ingest colostrum has been used to diagnose vertical BLV transmission. Two reports revealed 20% [21] and 4.8% [22], of neonates seropositive by Agar Gel Immunodiffusion test (AGID), and another study [23] detected in two herds 10% and 13% of neonates seropositive by AGID, and this type of cell has been observed in aborted bovine fetuses without conclusive differential diagnosis, which may indicate an infectious etiology [19,13].

Since bovine leukemia is prevalent in Brazilian cattle, and BLV in aborted bovine fetuses has not been investigated, the aim of this research was to investigate the infection of BLV in aborted fetuses using molecular techniques (nested-PCR, DNA sequencing, and phylogeny), ELISA, and examination of histological lesions suggestive of viral abortion, as well as examination of BLV positive fetuses for co-infection with other pathogens.

MATERIAL AND METHODS

This research was approved by the Bioethics Committee in Animal Research of the Biological Institute (CETEA-IB), registry number 122/12 according the Sociedade Brasileira de Ciência em Animais de Laboratório/Colégio Brasileiro de Experimentação Animal (SBCAL/COBEA).

Eighty bovine abortion cases from several Brazilian states were examined. Material comprised whole fetuses, fetal organs, and placentas. Materials were frozen or refrigerated and transported to the Centro de Pesquisa de Sanidade Animal do Instituto Biológico (CPSA-IB) from December, 2007 to October, 2012. When necropsy was performed by veterinarians in the field, the organs sampled varied, so sample numbers differed among tissues. The necropsies performed in an appropriate facility at CPSA-IB used sterilized instruments and the materials collected (organs, abomasal contents, thoraco-abdominal fluid) were placed in sterile bottles according to described protocols [25].

Estimates of the gestational age of fetuses were determined by one of three methods: crown-rump length [26], breeding dates, or estimates made by veterinarians.

Histology was performed on samples of thymus, spleen, lymph node, lung, heart, liver, kidney, adrenal gland, and brain fixed in 10% buffered formalin. Tissues were cut into small pieces, dehydrated, cleared, and embedded in paraffin; they were cut into the microtome (three µm) and stained with hematoxylin and eosin [27].

Thymus, spleen, lymph node, placenta, and thoraco-abdominal fluid were submitted to nested-PCR for BLV proviral DNA analysis. The DNA was extracted from chilled tissue samples using the commercial kit DNeasy Blood & Tissue Qiagen (Valencia, CA, USA). Amplification of the segment that encodes the env gp51 BLV gene used specific external primers for amplifying a segment of 598 base pairs (bp) (BLV1-5' TCT GTG CCA AGT CTC CCA GAT A 3' and BLV2-5' AAC AAC AAC CTC TGG TGA GGG T 3') and specific internal primers for amplifying a segment of 444 bp (BLV3-5' CCC ACA AGG GCG GCG GCG GTT T 3' and BLV4-5' GCG AGG CGC GGC GTT CAG GCT G 3') [28]. The positive control was a continuous lineage cell (fetal lamb kidney) infected with BLV and the negative control was VERO cells and ultra-pure water. Each sample was incubated in the thermocycler Mastercycler (Eppendorf, Foster City, CA, USA). The first amplification used 20 µL of GoTaq Green Master Mix (Cat #MT502, Promega, Madison, WI, USA) with 6.5 µL nuclelease-free water, 12.5 µL PCR buffer, 0.5 µL primer VL1B, and 0.5 µL primer VL2B for 5 µL of DNA. The PCR conditions of the first amplification were initial denaturation at 94°C for 2 min; 40 repeat cycles of denaturation at 95°C for 30 sec; annealing at 62°C for 30 sec, extension at 72°C for 1 min, and a final extension at 72°C for 4 min. The second amplification used 22 µL of GoTaq Green Master Mix (Cat #MT122, Promega, Madison, WI, USA) with 9.0 µL nuclelease-free water, 12.5 µL PCR buffer, 0.25 µL primer VL3B, 0.25 µL primer VL4B, and 3 µL of the final product of the first amplification, and each sample was incubated in the thermocycler. The conditions for the second amplification were denaturation at 94°C for 2 min, 40 cycles at 95°C for 30 sec, annealing at 70°C for 30 sec, extension at 72°C for 1 min, and a final extension at 72°C for 4 min. The analysis of amplified products was performed by electrophoresis (100 V/60 min) in a low melting point agarose gel (Aagarosis Cat. #V31125, LE, Promega, Madison, WI, USA) at 1.5% in Buffer TAE (Cat. #V4281, Promega, Madison, WI, USA) pH 8.2-8.4 1x. The DNA samples were stained with 1 µL Gel Red nucleic acid stain (Biotium Inc., Hayward, CA, USA) diluted in ultra-pure water at 1:150 and 2 µL of loading (6X DNA Loading Dye, Thermo Scientific, Waltham, MA, USA). A 100 bp DNA ladder (Fermentas, Vilnius, Lithuania) was used. The gel image under UV light (320 nm) was recorded using a system for photo-documentation and analysis (Alpha Innotech, San Leandro, CA, USA).

Eight samples yielded sufficient DNA for sequencing, they were purified with the Qiaquick PCR Purification kit 250 (cat. #28106, Qiagen, Valencia, CA, USA) and subjected to sequencing by chain termination with deoxynucleotides marked with fluorophores. The reaction was performed with 50 µL of the PCR product, 4 µL of each primer used in PCR (final concentration 3.2 µM) for a total...
RESULTS AND DISCUSSION

Nested PCR confirmed the presence of proviral DNA, showing BLV infection in 13 of the 80 fetuses (16.25%). This nested-PCR rate is similar to the seropositivity rates (AGID test) in newborn calves before colostrum ingestion [21-23,42]. The World Organization for Animal Health recommends the use of maternal and fetal blood before colostrum ingestion [21-23,42]. The World Organization for Animal Health recommends the use of maternal and fetal blood and abortion fluid for assessment of BLV pro-viral DNA by nested-PCR [37]. No sample of fetal thoraco-abdominal fluid was positive with ELISA, but two samples were nested PCR positive (Table 1). It was not possible to correlate results of indirect ELISA with those of nested PCR, since no fetus showed BLV antibodies with ELISA, indicating the importance of direct diagnosis of the pathogen using molecular techniques, which have greater sensitivity, since they detect animals in which BLV is in incubation or latency and that have not yet seroconverted [42-45].

Table 1: Distribution of 20 BLV-positive samples among lymphatic organs and thoraco-abdominal fluid identified by nested PCR, n=80 fetuses.

<table>
<thead>
<tr>
<th>Tissue and fluid samples</th>
<th>Number of cases</th>
<th>Number of positive tissues and fluids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoraco-abdominal fluid</td>
<td>58</td>
<td>2</td>
</tr>
<tr>
<td>Lymph node</td>
<td>56</td>
<td>3</td>
</tr>
<tr>
<td>Spleen</td>
<td>76</td>
<td>7</td>
</tr>
<tr>
<td>Thymus</td>
<td>76</td>
<td>8</td>
</tr>
</tbody>
</table>

BLV proviral DNA is most readily identified in the lymphatic system, tissues recommended by World Animal Health Organization for detection [37]. In the present study, nested-PCR identified BLV in 8 thymus, 7 spleen, and 3 lymph node samples, and thymus and spleen were the most frequently sampled lymphatic organs, each at 76 (Table 1). Most positive fetuses showed BLV in a single tissue: thymus in 4, followed by spleen in 3, and in thoraco-abdominal fluid in two. However, four fetuses tested positive in more than one tissue-type (Table 2). Thus the diagnosis of BLV increases with examination of multiple organs. It is known that antigens administered to a fetus intravenously are captured in liver, spleen, and bone marrow and the immune response will occur primarily in spleen and lymph nodes [46].

Table 2: Cases of multiple organ BLV-positive fetuses identified with nested PCR, n=13.

<table>
<thead>
<tr>
<th>Infection combinations in single and multiple organs</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>4</td>
</tr>
<tr>
<td>Spleen</td>
<td>3</td>
</tr>
<tr>
<td>Thoraco-abdominal fluid+spleen</td>
<td>2</td>
</tr>
<tr>
<td>Thoraco-abdominal fluid+spleen+thymus</td>
<td>1</td>
</tr>
<tr>
<td>Lymph node+thymus</td>
<td>1</td>
</tr>
<tr>
<td>Spleen+thymus</td>
<td>1</td>
</tr>
<tr>
<td>Lymph node + thymus</td>
<td>1</td>
</tr>
<tr>
<td>Thoraco-abdominal fluid</td>
<td>0</td>
</tr>
<tr>
<td>Lymph node</td>
<td>0</td>
</tr>
<tr>
<td>Thoraco-abdominal fluid+lymph node</td>
<td>0</td>
</tr>
<tr>
<td>Thoraco-abdominal fluid+lymph node+spleen+thymus</td>
<td>0</td>
</tr>
<tr>
<td>Lymph node+spleen</td>
<td>0</td>
</tr>
<tr>
<td>Lymph node+spleen+thymus</td>
<td>0</td>
</tr>
</tbody>
</table>

Positivity for BLV by nested-PCR was detected at 3 to 6 months gestation in 5 of 13 aborted fetuses and in 7 of 13 at 6 to 9 months. In one positive fetus the gestation was not determined. A study found in 5 of 15 seropositive fetuses (5 to 9 months) from cows with advanced lymphosarcoma [24]. This was corroborated by the present finding of vertical transmission of BLV from the second trimester. BLV intrauterine transmission in the first trimester of pregnancy was reported in up to 8% of seropositive cows, mainly...
in those with a high concentration of virus and low antibody titers [20]. This indicates that the fetus can be infected in utero, since in bi-angulates the sindesmocorial placenta is impermeable to maternal immunoglobulins, and bovine fetuses are unable to produce antibodies before 100 days. Immunoglobulins, when found in fetuses, are produced by their own immune system [46].

The majority of BLV positive fetuses were 3+ months into gestation, and no thoraco-abdominal fluid was positive for BLV antibodies. This may have been due to a) Viral titer level insufficient to provoke an immune response, according to a report where no antibody production was observed in BLV infection of less than 100 copies of proviral DNA/µg [47]; b) A deficient primary immune response in which the immune system does not recognize the viral antigen, since BLV may decrease IgM producing cells in lymph node and spleen [48]; c) The long period required for BLV incubation to seroconvert [15]; and d) Viral latency due a plasma blocking factor (PBPF) [49,50].

The sampling (80 cases) took place mainly in São Paulo with 39 fetuses and Minas Gerais State with 24. The BLV infection rate in São Paulo was 7 of 39 and, in Minas Gerais, 4 of 24, with a single positive case in the 5 samples from Goiás and in the 3 from Bahia. The highest frequency of fetuses BLV positive by nested-PCR was observed in dairy cattle, 8 of 13, in agreement with other studies [17].

Sequencing was possible in only 8 of the 13 cases positive for BLV because of the low amount of proviral DNA. This may be related to loss of DNA due to fetal autolysis, improper collection, preservation, and transportation of samples from the farm to the laboratory, or the unsatisfactory purification of the products sequenced. The 8 sequences had approximately 356 bp. The partial nucleotide alignment (356 nt) for the env gp51 gene of these sequences, but separated by longer branches [35]. The gp51 epitopes have high genetic variability that makes identification difficult.

The sequence LAP 54363B/SP presented similarity with JN254634 from Argentina [35] and the Brazilian JN254640 and JN254636 genotype 1 [33]. The sequence LAP 54363B/SP presented similarity with JN254634 from Argentina [35] and the Brazilian JN254640 and JN254636 genotype 1 [33].

Table 3: Sequences with maximum and minimum similarity of nucleotides in the consensus region, when compared with one another, with GenBank, and other Instituto Biológico isolated sequences.

<table>
<thead>
<tr>
<th>Similarity</th>
<th>Nucleotide identity</th>
<th>Maximum (100%)</th>
<th>Minimum (95.2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among the</td>
<td>LAP 54363B, LAP 54363B and</td>
<td>L54363B and L54363B and L56089T and L56089L 55704B</td>
<td></td>
</tr>
<tr>
<td>sequences of this</td>
<td>L56089T and L56089L 55704B</td>
<td>L57474B and L55704 L</td>
<td>L55704L, LAP54363B and LAP56089L</td>
</tr>
<tr>
<td>study</td>
<td></td>
<td>Maximum (100%)</td>
<td>Minimum (95.2%)</td>
</tr>
</tbody>
</table>

It is important to perform BLV molecular study in hosts, because the virus exhibits a diversity of genotypes characterized by similar sequences, but separated by longer branches [35]. The gp51 epitopes have high genetic variability that makes identification difficult. Several authors state that the molecular variations are responsible for differences between molecular and serologic diagnoses [51-54]. The sequence LAP 54363B/SP presented similarity with JN254634 found in São Paulo State (SP) [33], and belongs to genotype 5. The sample LAP 57474B from Minas Gerais State, when compared with the same cluster of the sample AF399702 from Minas Gerais [31] was also identified as genotype 5. Another study concluded that there was no correlation among sequences of Brazilian samples and viral pathogenicity and tissue tropism [32]. The present study suggests further research to investigate the association between BLV genotypes and clinical symptoms. Another study showed that, the sequence JN254639, genotype 6, has 100% similarity with LAP 55150T, both from São Paulo State [33]. Sample LAP 55704L is in the cluster of the sequences FM 209475 and FM 209469 (Uruguay) [34] and FJ808578 (Argentina) [35] with high bootstrap value (94) and belongs to genotype 1 (Figure 1, Table 3).

A phylogenetic tree (Figure 1) was constructed using the sequences of 356 (nt) from the env gp51 gene identified in the present study, sequences with major similarity recovered from GenBank, disposing access number, country of origin, and genotype [31-35], and other Brazilian strains VLB 05 to VLB 11 (Laboratório de Vroses de Bovídeos-Instituto Biológico-São Paulo-Brazil). The values of 1000 bootstrap replicates used the method of maximum composite likelihood.

A previous report [34] identified samples in Brazil as 1, 5, 6, and 7, as in the present study, and the Uruguay samples only as genotype 1, revealing specific geographic BLV clustering. Sequence LAP 56089T showed maximum similarity to the Brazilian sample JN254634 genotype 5, and samples LAP 57095PL and 55150T presented a bootstrap value 95% in the cluster of the Brazilian sample JN254634, genotype 6 (Figure 1, Table 3).

The histological changes observed in BLV-positive fetuses...
were hyperplasia of thymus, spleen, and lymph node; and mononuclear inflammatory infiltrate in brain, lung, heart, liver, kidney, adrenal gland, and placenta, characterizing a generalized lymphoproliferative reaction and suggesting a cellular reaction to an infectious agent. Infection by pathogens has moderate effects in the cow but may be lethal to a fetus, with the fetal infection inducing lymphoid hyperplasia and a rise in immunoglobulins [46]. Histological alterations in lymphoid organs were observed in both BLV negative and positive fetuses. BLV positive fetuses presented hyperplasia in all thymus samples (n=10), 7 of 8 lymph nodes, and in white pulp of all of 13 spleen samples. BLV negative samples showed hyperplasia in 38 of 53 thymus samples analyzed, 20 of 30 lymph nodes, and in white pulp of 23 of 28 spleen samples. BLV positive fetuses presented mononuclear inflammatory infiltrate in all liver samples (n=10), 11 of 13 lung samples, 4 of 12 heart samples, 8 of 10 adrenal glands, 4 of 8 kidneys, and 9 of 11 brain samples. BLV negative fetuses presented the same type of mononuclear infiltrate in 19 of 21 liver samples, 39 of 47 lung samples, 34 of 47 heart samples, 14 of 28 adrenal gland samples, 12 of 39 kidney samples, and 28 of 34 brain samples. All BLV positive fetuses presented mononuclear inflammatory infiltrate in at least one organ, and this was also true of 64 of the 67 BLV negative fetuses, suggesting infectious abortion associated with other pathogens. Other researchers observed these lesions at high rates in aborted fetal tissues [1,9,13] and the necropsy of BLV seropositive pregnant cows with lymphosarcoma showed two fetuses with neoplastic lesions of organs and reactive lymph nodes [24].

Figure 1: Phylogenetic tree developed by the neighbour-joining method with the substitution pattern maximum composite likelihood. The numbers at each node represent the values of 1000 bootstrap replicates. BLV sequences recovered from GenBank used for genealogy, showing the accession number, country of origin, and genotype of the env gene [31-35].

Tissue lesions suggestive of infection were observed in at least one organ of all tested fetuses. It is possible that both BLV and other pathogens were the source of the mononuclear inflammatory infiltrate in organs, as well as the reactivity of lymphoid organs, in all the fetuses the differential diagnosis was done for other pathogens: in 3 of 13 positive fetuses there was co-infection of BLV and *Klebsiella* spp., *Trueperella (Arcanobacterium) pyogenes* and *Streptococcus* spp, each identified in a single fetus. Pathogens were observed in 12 of 67 BLV negative fetuses: *Neospora caninum* in 4, BVD virus in 3, *Escherichia coli* in 2, and single instances of *Trueperella (Arcanobacterium) pyogenes*, *Bacillus abortus*, and *Staphylococcus aureus*. No *Leptospira* spp. or BoHV-1 was detected (Table 4). In 55 of the fetuses it was not possible to detect any pathogen, a rate similar to that reported in Brazil [10-12], while another study [9] detected specific abortion pathogens in 46.7% of 490 aborted bovine foetuses.
The low rate of brucellosis (1/80) in aborted foetuses relects the Brazilian program to combat bovine brucellosis PNCEBT [54] conducted in the state of Sao Paulo [55] and Minas Gerais [56], which were the source of the majority of samples in this study. With the eradication of brucellosis, differential diagnosis should include the potential pathogens to expand a bovine abortion sanitary program, in addition to developing more specific and sensitive laboratory testing techniques [2,5,9,11,12,13]. The main factors that interfere with the success of laboratory analysis appear to be fetal autolysis, inadequate sampling of fetal organs, and lack maternal and fetal serum and placenta [6]. It is necessary to educate veterinarians about procedures for fetal necropsy, sampling, preser- vation, and transport of samples to the laboratory [25].

The low number of BLV positive samples infected or no by other pathogens (Table 4) did not perform statistical analysis, in order to understand if there were significant differences among infected and infected BLV fetuses. Since retroviruses are immunosuppressive [15,57,58], further studies with a bigger sampling are needed for elucidating BLV pathogenesis and pathogen interactions. Anim Reprod Sci. 2000;60:131-143.

In relation to BLV interference in cow reproduction, several author could not prove statistically significant differences when comparing groups of seroreagent cows with non-seroreagents, regarding the following parameters: age at first calving [59,61] and number of services [62]. In contrast, Brenner et al. [63] showed that there was a greater interval between calving in BLV seroreagent cows. Since BLV seropositive rates in Brazilian cattle herds are elevated and the virus is spread throughout the territory [17], the rates of BLV transmission show the necessity of prophylactic measures in Brazilian cattle herds, in order to avoid infection in utero.

CONCLUSION

Considering that BLV causes leukemia and lymphosarcoma in young and adult animals, and that this virus can cause immunosuppression and vulnerability to pathogens that can cause abortion, the BLV transplacental transmission rates in Brazilian herds show the necessity for preventive programs to avoid fetal infection. Further studies with a bigger sampling are needed to understand whether BLV is a causative or predisposing agent of bovine abortion.

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ACKNOWLEDGEMENTS

Thanks to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, São Paulo, Brazil) for Research Grant processes 2009/13864-8 and 2012/01033-7. To FAPESP for TT-3 Scholarship (Capacitação técnica nível III): 2012/06276-5. To CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Brasil – código de financiamento 001), for Masters Grant.A.

REFERENCES


