DOI: 10.1590/1808-1657000092020

Causes of equine abortion, stillbirth, and perinatal mortality in Brazil*

Causas de abortamento, natimortalidade e mortalidade em equinos no Brasil

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ABSTRACT: Abortion and complications in reproduction are important causes of economic loss in horse breeding. Studies of its causal agents can help to identify the primary pathogens or other factors involved and define appropriate measures to reduce its occurrence. This research aimed to investigate the primary causes of equine abortion, stillbirth, and perinatal mortality in regions of Brazil. Tissue from aborted fetuses, stillbirths, neonates and foals submitted to the Biological Institute of São Paulo, Brazil, from January 2010 to July 2013 were processed for viral and bacterial isolation, polymerase chain reaction (PCR), histology, and immunohistochemistry. Bacterial infection was the primary detected cause of abortion, found in 16 of the 53 animals submitted for bacterial analysis followed by viruses analysis in 2 of 105 animals, and noninfectious causes (neonatal isoerythrolysis) in 2 of 105 animals. Fungi were found in a single sample of 53 tested. The most frequent bacteria recovered were *Escherichia coli*, *Enterobacter aerogenes*, combined *E. coli* and *Streptococcus* spp., *Staphylococcus* spp., and *Bacillus* spp. The following agents were each observed in a single sample: *Arcanobacterium pyogenes*, *Streptococcus* spp., *Corynebacterium* spp., *Actinobacillus* spp., and *Rhodococcus equi*. The predominant identification of fecal and other opportunistic bacteria as opposed to pathogens commonly associated with equine abortion, such as *Leptospira* spp. and equine herpesvirus type 1 (EHV-1), suggests the need of improving hygiene management of breeding mares to prevent bacterial infection that may cause fetal loss, stillbirth, and perinatal mortality.

KEYWORDS: *Equus caballus*; infectious diseases; reproduction.

RESUMO: Abortamento e complicações na reprodução são importantes causas de perda econômica na equideocultura. Estudos dos agentes causais podem ajudar a identificar patógenos ou outros fatores envolvidos e definir medidas apropriadas para reduzir sua ocorrência. Esta pesquisa investigou as causas primárias de aborto, natimortalidade e mortalidade perinatal em equinos de diversas regiões do Brasil. Tecidos de fetos abortados, natimortos e potros submetidos ao Instituto Biológico de São Paulo, Brasil, no período de janeiro de 2010 a julho de 2013, foram processados por meio de técnicas de isolamento viral e bacteriano, PCR, histologia e imuno-histoquímica. Infecção bacteriana foi a causa mais detectada, encontrada em 16 de 53 amostras submetidas à análise bacteriana, seguida de causa viral em 2 de 105 amostras, e causas não infecciosas (isoeritrólise neonatal) em 2 de 105 amostras. Fungo foi encontrado em uma única amostra de 53 testadas. As bactérias isoladas mais frequentemente foram *Escherichia coli*, *Enterobacter aerogenes*, *E. coli* associada a *Streptococcus* spp., *Staphylococcus* spp. associado a *Bacillus* spp. Os seguintes agentes foram observados em uma única amostra cada: *Arcanobacterium pyogenes*, *Streptococcus* spp., *Corynebacterium* spp., *Actinobacillus* spp. e *Rhodococcus equi*. A identificação predominante de bactérias fecais e outras bactérias oportunistas, ao invés de outros patógenos comumente associados a quadros de abortamento equino, tais como *Leptospira* spp. e Herpesvírus equino tipo 1, sugere a necessidade de maior atenção no manejo higiênico das éguas em reprodução, a fim de prevenir infecções bacterianas que possam causar perda fetal, natimortalidade e mortalidade perinatal.

PALAVRAS CHAVES: *Equus caballus*; doenças infecciosas; reprodução.

*This article is part of the master's dissertation of the first author.

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Received on: 03/04/2020. Accepted on: 08/02/2020

INTRODUCTION

Abortion and complications of reproduction are important causes of economic losses in equine breeding due to the elevated costs incurred with diagnosis and treatment as well as the loss of animals (MOREIRA et al., 1998).

The causes of equine abortion, stillbirth, and perinatal mortality include bacterial agents *Streptococcus* spp., *Staphylococcus* spp., *Escherichia coli, Leptospira* spp., *Rodococcus equi*, and *Klebsiella* spp. (GENOVEZ et al., 1995; LAUGIER et al., 2011); viral agents as equid alphaherpesvirus 1 and equine viral arteritis (EVA) (MOREIRA et al., 1998; LAUGIER et al., 2011; HONG et al., 1993); and fungi *Aspergillus* spp., *Candida albicans*, *Mucor* spp., *Zygomycetes* spp., and *Histoplasma capsulatum* (HONG et al., 1993; SMITH et al., 2003; SZEREDI et al., 2008; JUFFO, 2016).

Birth of twins has been considerably reduced through detection with ultrasound scans, but other noninfectious causes including neonatal isoerythrolysis (an immune condition that leads to the lysis of blood cells after ingesting of antibodies present in colostrum), umbilical cord torsion, and umbilical cord/cervical pole ischemia disorder must be considered (HONG et al., 1993; SZEREDI et al., 2008; RICKETTS et al., 2001; RIZZONI; MIYAUCHI, 2012).

Studies of causes of abortion, stillbirth and perinatal mortality can help to identify the primary pathogens or other factors involved and define appropriate measures to reduce its occurrence. The most recent report from Brazil was conducted from January 2000 to June 2011 and presented data from a single region of the country (MARCOLONGO-PEREIRA et al., 2012). Further research is needed to expand available information. The goal of the present study was to determine the primary causes of equine abortion, stillbirth, and perinatal mortality in Brazil.

MATERIAL AND METHODS

Sampled animals

Convenience samples of *Equus caballus* were obtained from 37 fetuses (fetal loss before 300 days gestation), three stillbirths (death after 300 days),and perinatal deaths (death within seven days of birth), 21 foals under one month of age, and 44 fetuses of unknown age, from a total of 105 animals sent to the Biological Institute of São Paulo, from January 2010 to July 2013.

Tissue samples

Samples were submitted to the Pathological Anatomy Laboratory of the Biological Institute of São Paulo for differential diagnosis of abortion causes. Refrigerated tissues were subjected to virus isolation and polymerase chain reaction (PCR) performed at the Rabies and Encephalitis Laboratory and to bacteriological analysis by the General Bacteriology Laboratory and Reproductive Bacterial Diseases Laboratory. Tissue fragments were fixed in 10% buffered formalin for histology and immunohistochemistry (IHC) at the Pathological Anatomy Laboratory.

Tissue samples submitted to the laboratory or collected during laboratory necropsy included central nervous system (CNS) (n = 44), lungs (n = 83), heart (n = 67), liver (n = 82), kidney (n = 86), spleen (n = 74), thymus (n = 26) stomach contents (n = 23), placenta (n = 40), and umbilical cord (n = 16).

Histology was conducted according to standard protocols (PROPHET et al., 1995). Tissues were fixed in 10% buffered formalin for 48 h, transferred to 70% ethanol, dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin. Sections were cut at 3 µm, deparaffinized, rehydrated, and stained with hematoxylin and eosin.

Immunohistochemistry (IHC)

Sections of lung and brain (3µm) for detection of equine herpesvirus type 1 (EHV-1) and of liver and kidney (3 µm) for *Leptospira* spp. were dewaxed in xylene 30 min at 37 °C followed by block of endogenous peroxidase in solution of 20 mL of 30% hydrogen peroxide diluted in 80 mL methanol for 30 min. Antigen retrieval was performed with citrate buffer (pH 6.0) by heating in a 1000 W microwave oven for 15 min prior to incubation with a 1:1000 dilution of goat antiserum (VMRD USA Inc. catalog 210-70-ERV, Pullman, Washington, USA) specific for ERV/EHV-1 (SILVA et al., 2018) and primary antibody to *Leptospira* serovar Canicola, strain LO4, titer 12:400 (FAINE, 1994). Sections were incubated with biotinylated secondary antibody (anti-mouse, -rabbit, and -goat immunoglobulin) (LSAB+ System -HRP, Dako ref. K0690 – Dako Cytomation, Carpinteria, California, USA) and exposure to 3,3'diaminobenzidinechromogen solution (DAB – Dako code ref K3468), counterstained with Mayer's hematoxylin, and mounted with Entellan (Merck) synthetic resin.

Virus isolation

One mL of 20% organ suspension [2 g of each organ tissue macerated with 8 mL Eagle's minimum essential medium (EMEM)] was inoculated into a monolayer culture of Vero cells at 37 °C for 1 h (SILVA et al., 2018). At the end of the attachment period, cell monolayers were rinsed with 6 mL maintenance medium (EMEM containing 2% fetal calf serum supplemented with 200 U/mL penicillin, 200 mg/mL streptomycin, and 50 U/mL nystatin) and incubated at 37 °C. Three blind passages were conducted at seven-day intervals, and cells were assessed for herpesvirus cytopathic effect characterized by the presence of rounded, retractile cells, lysis, or syncytia formation.

Bacterial culture

A pool of organ tissue from each animal (n = 53) and 23 stomach contents were submitted to isolation and identification of aerobic (*Enterobacteria* and Gram-positive cocci) and microaerophilic bacteria (*Campylobacter* spp., *Brucella* spp., *Leptospira* spp., *Listeria monocytogenes*) (GENOVEZ et al., 1995; KIRKBRIDE, 1990; OIE, 2015). Microorganisms isolated in pure culture or predominating were considered potential causes of abortion (KIRKBRIDE, 1990).

Culture methods used for *Leptospira* spp. were Ellinghausen-McCullough-Johnson-Harrismedium (Difco, USA) and Fletcher's medium with 15% sterile rabbit serum inactivated at 56 °C for 30 min, enriched with 1% 5-fluorouracil, 400 mg/L (Sigma, USA).

Polymerase chain reaction (PCR)

DNA was extracted using TRI Reagent according to the manufacturer's protocol. from 20% fetal organ suspensions (CHOMCZYNSKI, 1993). To detect EHV-1, primers that hybridize to the highly conserved gB gene regions inner primers (Invitrogen Brazil Ltd., São Paulo, Brazil) P1 forward 5'-CTTGTGAGATCTAACCGCAC-3'/P2 outer reverse 5'-GGGTATAGAGCTTTCATGGG-3' and P1/P3 inner reverse 5'-GCGTTATAGCTATCACGTCC-3' (MORI et al., 2009) were used.

Fragments of organs (~2 g) were macerated in a homogenizer (Stomacher 80) to obtain a pool of organ tissue from each animal, suspended in TE buffer (10% w/v), and stored at -20 °C until DNA extraction. Polymerase chain reaction to diagnose *Leptospirosis* employed primers specific to *Leptospira* spp. on a 331 bp fragment (Lep 1: 5 'GGCGGCGCGTCTTAAACA TG 3' and Lep 2: 3 'TTC CCCCCATTGAGCAAGATT 5').

RESULTS

Of the 105 animals examined, the cause of death could not be established in 84. Two showed a noninfectious cause (neonatal isoerythrolysis), and two were positive for EHV-1 by PCR. Tests for *Leptospira* spp. by isolation, PCR, or IHC were all negative. Bacteriological differentiation from *Leptospira* spp. was performed on 53 of the 105 samples, of which16 were positive for other bacterial agents and one for yeast (Table 1).

Bacteria isolated in pure or predominant culture were *E. coli (*4/53), *Enterobacter aerogenes (*3/53), mixed *E. coli* and *Streptococcus* spp. (2/53), mixed *Staphylococcus* spp. and *Bacillus* spp. (2/53), and a single instance each of *Arcanobacterium pyogenes*, *Streptococcus* spp., *Corynebacterium* spp., *Actinobacillus* spp., *Rhodococcus equi*, and an unspecified yeast (Table 2).

In the 17 horses that tested positive for bacteria and fungi of the 53 examined, the main pathological lesions were white pulp hyperplasia in 12 of 12 spleen samples; lymphoid hyperplasia in 6 of 7 thymus samples; nonpurulent meningoencephalitis in 6 of 7 of CNS samples; nonpurulent pneumonia in 9 of 14 lung samples; nonpurulent glomerulonephritis in 7 of 12 kidney samples; nonpurulent hepatitis in 7 of 15 liver samples and nonpurulent myocarditis in 5 of 54 cardiac tissue samples; 84 animals were negative bacteria and fungi analysis also but presented predominant splenic white pulp hyperplasia in 58 of 64 spleen samples; thymic lymphoid hyperplasia in 15 of 19 thymus samples; nonpurulent meningoencephalitis in 23 of 37 CNS samples; nephrosis in 41 of 74 kidney samples; nonpurulent hepatitis in 29 of 67 liver samples; nonpurulent pleuropneumonia in 25 of 70 lung samples; and nonpurulent myocarditis in 5 of 54 heart samples 9.3% (5/54).

Table 2. Bacteria species, plus a single yeast, isolated from 53 horse guts from aborted fetuses, and perinatal mortalities.

Isolated agent	Horses n (%)
Escherichia coli	4 (7.5)
E. coli + *Streptococcus* spp.	2 (3.8)
Enterobacter aerogenes	3 (5.7)
Staphylococcus spp. + *Bacillus* spp.	2 (3.8)
Actinobacillus spp.	1 (1.9)
Arcanobacterium pyogenes	1 (1.9)
Corynebacterium spp.	1 (1.9)
Rhodococcus equi	1 (1.9)
Streptococcus spp.	1 (1.9)
Yeast fungus	1 (1.9)
Negative samples	36 (67.9)
Total	53

Table 1. Cause or causal agent classification of equine abortion, stillbirth, and perinatal mortality.

Cause of abortion/ perinatal death	Cases (n)	Samples analyzed (n)	Frequency (%)
Noninfectious (neonatal isoerythrolysis)	2	105	1.9
Viral infection (EHV-1)	2	105	1.9
Bacterial infection	16	53	30.2
Fungal infection	1	53	1.9
No diagnosis	84	105	80.0

Forty percent of the analyzed samples did not have the time of abortion or death reported on the submission form, including the two EHV-1 positive samples. Of the animals positive for bacterial infections, the abortions occurred most frequently at 4-6 months gestation followed by 6-9 months. Two stillborn showed a noninfectious cause (neonatal isoerythrolysis) (Table 3). One of the two fetuses positive for EHV-1 was from São Paulo State and the source of the other was not provided. Among fetuses positive for bacteria, the highest number came from the states of São Paulo and Paraná with seven each (Table 4).

DISCUSSION

Of the 105 aborted fetuses, stillbirths and perinatal deaths submitted for differential diagnosis of causes of abortion or perinatal mortality, 53 were submitted to bacteriological analysis with 16 found positive for bacterial agents representing 30.0% of those tested. Other studies point to bacterial infections as the cause of 20–34% of equine infectious abortions, representing an important economic loss (MOREIRA et al., 1998; GENOVEZ et al., 1995; LAUGIER et al., 2011; MARCOLONGO-PEREIRA et al., 2012). In the present study, the predominant agents isolated were *E. coli* in 4 of the 16 positive samples, *E. aerogenes* (3/16); *E. coli* + *Streptococcus* spp. (2/16); *Staphylococcus* spp. + *Bacillus* spp. (2/16).

In Brazil in the 1990s, equine bacterial abortion was predominantly associated with beta-hemolytic *Streptococcus* spp., *E. coli*, *R. equi*, *S. aureus*, and *Staphylococcus* spp. (MOREIRA et al., 1998; GENOVEZ et al., 1995). Other bacteria such as *Salmonella* spp., *L. monocytogenes*, and *Klebsiella oxytoca* were also isolated at lower frequency (GENOVEZ et al., 1995). The most recent published data in southern Rio

Table 3. Cause of death data on 105 aborted horse fetuses, stillbirths, and perinatal mortalities by age.

Gestation age	Viruses n (%)	Bacteria n (%)	Fungi n (%)	Noninfectious disease n (%)	Undiagnosed n (%)
Fetus 1-3 months	0	0	0	0	6 (5.7)
Fetus 4-6 months	0	7 (6.6%)	0	0	3 (2.8)
Fetus 7-9 months	0	3 (2.8%)	0	0	11 (10.4)
Fetus > 9 months	0	3 (2.8%)	0	0	4 (3.8)
Stillbirth	0	0	0	2 (1.9)	1 (0.9)
Month old foal	0	1(0.9)	0	0	20 (19.0)
Not available	2 (1.9)	3 (2.8)	1 (0.9)	0	38 (36.1)
Sub-total	2 (1.9)	17 (16.1)	1 (0.9)	2 (1.9)	83 (79.0)
Total			105		

Table 4. Aborted horse fetuses, stillbirths, and perinatal mortalities classified by state of origin.

State of origin	Viruses (%)	Bacteria n (%)	Noninfectious causes* n (%)	Undiagnosed n (%)
Minas Gerais	0	0	0	8 (7.6)
Distrito Federal	0	1 (0.9)	0	0
Mato Grosso do Sul	0	0	1(0.9)	1 (0.9)
Pará	0	0	0	1 (0.9)
Sergipe	0	0	0	1 (0.9)
Tocantins	0	0	0	1 (0.9)
Paraná	0	7 (6.6)	0	10 (9.5)
Rio Grande do Sul	0	1 (0.9)	0	2 (1.9)
Santa Catarina	0	0	0	1 (0.9)
Rio de Janeiro	0	0	0	9 (8.5)
São Paulo	1 (0.9)	7 (6.6)	0	44 (41.9)
Not informed	1 (0.9)	1 (0.9)	1 (0.9)	6 (5.7)
Subtotal	2 (1.9)	17 (16.1)	2 (1.9)	84 (80.1)
Total		105		

*Isoerythrolysis.

Grande do Sul reported beta-hemolytic *Streptococcus*, *Klebisiella pneumoniae*, *E. coli* and *Streptococcus* spp. to be predominant (MARCOLONGO-PEREIRA et al., 2012).

In the USA, studies of causes of equine abortion, stillbirth, perinatal mortality, and placentitis have predominantly shown isolation of *Streptococcus equi* subspecies *zooepidemicus*, *E. coli*, and alpha- and beta-hemolytic *Streptococcus* (HONG et al., 1993; TENGELSEN et al., 1997). Other agents including *Actinobacillus equuli*, *Serratia marcescens*, *R. equi*, *S. equi*, *S. equisimilis*, *Campylobacter* spp., *Corynebacterium* spp., *Klebsiella*, *Micrococcus*, *S. aureus*, and *Staphylococcus xylosus* have also been isolated (TENGELSEN et al., 1997).

Similarly, in the United Kingdom, Hungary, France, and Italy, *E. coli*; *Streptococcus* spp., mainly beta-hemolytic and *zooepidemicus*; and *Staphylococcus* spp. were the most common bacteria isolated with *Klebsiella pneumoniae, E. aerogenes* and *Chlamydophila psittaci* also mentioned (LAUGIER et al., 2011; SMITH et al., 2003; SZEREDI et al., 2008; MARENZONI et al., 2012). In Japan, in 2007–2008, *S. equi* subsp. *zooepidemicus* were the most frequently isolated bacteria (29.5%), followed by *E. coli* (20.5%) and *Salmonella abortus equi* (MURASE et al., 2017).

LAUGIER et al. (2011) determined the bacterium responsible for equine abortion could be isolated from allantochorion and fetal organs, implying fetal septicemia leading to chronic placental insufficiency and extensive placental lesions, which can retard fetal development or result in emaciation and death of the fetus.

In the present study, none of the fetuses were positive for *Leptospira* spp. Low rates of *Leptospira* spp. as a cause of equine abortion or stillbirth death have been reported in Brazil: 1.88% (2/106) (GENOVEZ et al., 1995) and 0.0% (MOREIRA et al., 1998), with some evidence of higher rates: 5.6% (4/72) (MARCOLONGO-PEREIRA et al., 2012) and 5.2% (4/77) (JUFFO, 2016). Conversely, in an area of central Kentucky, USA, a peak of 15.7% of *Leptospira* spp. was reported by HONG et al. (1993) with serovars Pomona and Grippotyphosa predominating. Recent studies in other countries have revealed 0% to 1.9% (LAUGIER et al., 2011; RICKETTS et al., 2001; MARENZONI et al., 2012). Opportunistic fungi as cause of equine abortion are rare compared to bacteria (HONG et al., 1993; JUFFO, 2016; TENGELSEN et al., 1997). In the present study, a single yeast fungus, which could not be identified, was isolated from samples of liver and kidney of an aborted fetus. A similar rate (1/106) in Brazil was reported by GENOVEZ et al. (1995) with isolation of *Candida* spp. Other authors, in other countries report the isolation of *Mucor* spp. (1/77), *Zygomycetes* spp., and *H. capsulatum* (2/96) (SZEREDI et al., 2008; JUFFO, 2016; TENGELSEN et al., 1997). SMITH et al. (2003) reported fungal placentitis in 1.4% (18/1252) of cases with isolation of *Aspergillus* and *Absidia*.

Equid alphaherpesvirus 1 is traditionally recognized as an important agent involved in equine abortion. Levels of EHV-1 abortion reported in the 1990s varied from 8.3 to 40% (TENGELSEN et al., 1997; BAŻANÓW et al., 2014; SCHULTHEISS et al., 1993). More recent studies have pointed to lower rates of EHV-1: 6.9% in France (LAUGIER et al., 2011), 6.5% in the United Kingdom (SMITH et al., 2203), 16% in Hungary (SZEREDI et al., 2008), 10.1% in Japan (MURASE et al., 2017) and 8.9% in Germany (WEBER et al., 2018). A single study in Italy reported EHV-1 above 20% (MARENZONI et al., 2012).

In Brazil, since the 1990s, a similar decrease has been reported in rates of abortion due to EHV-1: 8.0% (3/50) (MOREIRA et al., 1998), 4.2% (3/26) (MARCOLONGO-PEREIRA et al., 2012), and 1.9% (2/105) (SILVA et al., 2018). BROWN et al. (2007) reported low prevalence of EHV-1 infection even among a population of thoroughbreds in which the virus was known to be endemic and suggested the possibility that pregnant mares become infected without aborting; however, it is important to mention that EHV-1 occurs more frequently in the final third of pregnancy. In the present study, the number of abortions with a gestational age of 10 to 12 months was small, which may influence in the low occurrence observed.

A study covering 1977–2010 in Poland found rates of 25.6% for EHV-1 (BAŻANÓW et al., 2014). The authors also report that the rates of isolation of the two viruses were inversely proportional, but most laboratories do not usually include them in routine differential diagnosis of equine abortion or foal death.

Two cases of death due to neonatal isoerythrolysis, an isoimmune-hemolytic anemia directly linked to the ingestion of milk were observed. The disease occurs when the maternal alloantibodies to the neonate blood are transferred to the foal via colostrum, causing lysis of red blood cells (RADOSTITS et al., 2002). No cases of umbilical cord torsion, dystocia, twinning, or other noninfectious cause were observed in the present study. In Brazil, MOREIRA et al. (1998) reported twinning (4.5%) as the only cause of noninfectious abortion in their study. MARCOLONGO-PEREIRA et al. (2012) found 8.3% of abortions to be noninfectious and mentioned umbilical cord torsion (2.8%), dystocia (1.4%), noninfectious maternal disease (1.4%), maternal-fetal incompatibility (1.4%), and congenital malformation (1.4%) as the main causes (MARCOLONGO-PEREIRA et al., 2012).

In the USA (TENGELSEN et al., 1997), United Kingdom (SMITH et al., 2003), France, (LAUGIER et al., 2011), and Japan (MURASE et al., 2017), high rates of noninfectious causes of abortion or stillbirth are reported. In the study of TENGELSEN et al. (1997), congenital defects including cranial and cervical spine and thoracic-lumbar deformities, as well as limb contractures accounted for 14.2% of congenital defects. SMITH et al. (2003), in the UK, reported high rates (38.8%) of problems associated with umbilical cord torsion and umbilical cord/cervical pole ischemia disorder as the most common diagnosis in noninfectious abortion and neonatal.

SMITH et al. (2003) also reported intrapartum stillbirth (13.7%) and twinning (6.0%).

LAUGIER et al. (2011), in France, reported 27.2% noninfectious causes, with umbilical cord disorders responsible for 16.5% of the cases, followed by placental villous hypoplasia or atrophy (4.7%), lethal congenital anomalies (1.9%), and twinning (1.5%). MURASE et al. (2017), in Japan, observed predominant noninfectious causes of abortion to be circulation failure (19.0%), followed by multiple causes (3.1%), deformity (0.6%), placental abnormality (0.6%), and other causes (0.3%).

Deformities including diaphragm aplasia, head deformity, insufficient abdominal wall closure, diaphragmatic aplasia, *Schistosomus reflexus*, severely deformed kidney and liver, severe placental calcification, and trauma of the mare have also been reported at low rates (SZEREDI et al., 2008; MURASE et al., 2017; TENGELSEN et al., 1997). In equine abortion samples analyzed by TENGELSEN et al. (1997), 46.0% of the animals presented deficiency in vitamin E, 80.1% were deficient in selenium, and 56.3% were deficient in both. The influence of these types of deficiency should be investigated as a noninfectious cause of equine abortion and stillbirth, since little research in the area has been published. In the present study, 80.0% of abortions showed no conclusive diagnosis.

Other authors have reported rates of uncertain diagnosis ranging from 25.1 to 47.2% (LAUGIER et al., 2011; SZEREDI et al., 2008; MARCOLONGO-PEREIRA et al., 2012), but SMITH et al. (2003) reported only 7.7% undetermined diagnosis. Often, uncertainty is likely due to advanced states of autolysis of the fetus or to fetuses submitted without associated membranes (LAUGIER et al., 2011; SMITH et al., 2003). Extensive autolysis was observed in samples of kidney, liver, and central nervous system. Nearly half of the studied samples were not submitted to bacteriological analysis since it was not requested by the owner/professional, which may have contributed to the high number of inconclusive cases.

Current literature confirms that fetal death can occur as a result of action of a microorganism on the fetus, as well as due to the influence of toxins and changes in fetal circulation via acute infectious processes generated by pathogens acting on the pregnant female (maternal septicemia). It is imperative for accurate determination cause of abortion that serological samples from the mother, placenta, umbilical cord, and vaginal or uterine discharge after delivery be examined, but this material is often not submitted to the laboratories (GENOVEZ et al., 1995; LAUGIER et al., 2011; SMITH et al., 2003; SZEREDI et al., 2008).

In practice animals sent to laboratories for necropsy often arrive after unfavorable storage conditions making diagnosis by conventional techniques a challenge. In addition, the clinical-epidemiological records are often incomplete. In the present study, age information was not available for most part of the samples. Current literature reports 85.4–94.3% of abortions to occur at 6–9 months of gestation, but earlier abortions may be underestimated because of difficulty in finding small fetuses in pasture and the possibility of their consumption by carnivores (LAUGIER et al., 2011; HONG et al., 1993; SMITH et al., 2003; MARCOLONGO-PEREIRA et al., 2012; SILVA et al., 2018; MURASE et al., 2017; TENGELSEN et al., 1997). Following recommended procedures in collection and transport of samples to the laboratory and the adequate filling of clinical-epidemiological records are imperative to the effectiveness of the differential diagnosis (OIE, 2015; PITUCO et al., 2010).

The main lesions in the animals with no diagnosis were in the lymphoid organs, splenic white pulp hyperplasia, thymic lymphoid hyperplasia, and in the central nervous system, nonpurulent meningoencephalitis, suggesting a neurotropic infectious agent. The most frequent pathology seen in kidney of samples positive for bacterial agents was nonpurulent glomerulonephritis, while in the negative bacteriological samples, no diagnosis concluded and the two cases positive for EHV-1, the predominant kidney lesion was nephrosis. Other studies of bacterial infection have reported predominant purulent suppurative and pyogranulomatous pneumonia or hepatitis and did not observe changes in kidney (LAUGIER et al., 2011; SZEREDI et al., 2008; JUFFO, 2016).

LAUGIER et al. (2011) reported 27 cases of abortion, including 24 with placentitis, in which macroscopic and/or histologic lesions observed were suggestive of an infectious origin but no specific pathogenic agent was isolated. In contrast, SZEREDI et al. (2008) and MARCOLONGO-PEREIRA et al. (2014) observed only 2% of cases with inflammatory lesions to have no identified agent. Described lesions were mild to moderate pneumonia, mild serous hepatitis, and nonpurulent nephritis. The low frequency of occurrence of EHV-1 and absence of *Leptospira* spp. can be attributed to antibodies due to natural infection and/or to the increased practice of systematic vaccination for these diseases in recent years, conferring maternal immunity (MARCOLONGO-PEREIRA et al., 2014; CARVALHO et al., 2000). Analysis of maternal blood serum together with the fetus and placenta is important to confirm this hypothesis, but serum of the mare is seldom referred for serodiagnosis (SILVA et al., 2018; BROWN et al., 2007).

To increase accurate detection of causes of abortion and perinatal mortality, it is also important to include in routine investigation other agents that have frequently been cited as causes of equine abortion, such as EHV-2 -4 and -5 equine infectious anemia, EVA, and *Chlamydia* (LAUGIER et al., 2011; SMITH et al., 2003; BROWN et al., 2007; SZEREDI et al., 2008; MARENZONI et al., 2012; BAŻANÓW et al., 2014; WEBER et al., 2018). Protozoan infections such as *Babesia equi*, *Encephalitozoon cuniculi, Neospora* spp., and *Toxoplasma gondii* are also often not considered causes of abortion, which may lead to inaccurate diagnosis of the cause of decreased birth rate in farm animals (SZEREDI et al., 2008; MARCOLONGO-PEREIRA et al., 2012).

Pasteurella pneumotropica and toxic plants such as *Ateleia glazioviana* are less commonly cited causes of equine abortion and placentitis and must be better researched (JUFFO, 2016, MARCOLONGO-PEREIRA et al., 2014).

The most frequently identified causes of abortion, stillborn, and perinatal mortality are still opportunistic bacteria often isolated from the gastrointestinal and genitourinary tract of mares and the environment (MOREIRA et al., 1998; GENOVEZ et al., 1995; JUFFO, 2016; MARCOLONGO-PEREIRA et al., 2012). The structure of the mare genital tract makes females more susceptible to infection, defects of conformation of vulva, loss of muscle mass of the perineum, as well as displacement of the anal sphincter anteriorly during estrus may increase this susceptibility (GRÜNERT et al., 2005). Despite improvements in environmental and nutritional management in horse rearing, the relative percentage of enterobacteria and Gram-positive cocci can indicate the need to reinforce hygiene measures in the management of pregnant mares, including maintaining the environment and facilities, as well as sanitation of utensils and systematic vaccination, to prevent fetal loss and perinatal mortality (MOREIRA et al., 1998). The high number of samples with no conclusive diagnosis highlights the need for greater care in submitting laboratory samples and the need to investigate causes of equine abortion in addition to those routinely researched.

CONCLUSION

The predominant identification of fecal and other opportunistic bacteria suggests the need of improving hygiene management of breeding mares to prevent bacterial infection that may cause fetal loss, stillbirth, and perinatal mortality, but more studies, which include a larger number of samples and pathogens not commonly investigated, are needed to support this theory.

ACKNOWLEDGEMENTS: Not applicable.

FUNDING: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) Finance Code 001 and the Fundação de Amparo à Pesquisa do Estado de São Paulo (Grant: 2009/13864-8, 2012/00277-0).

CONFLICTS OF INTEREST: All authors declare that they have no conflict of interest.

ETHICAL APPROVAL: This study was approved by Ethics Committee on Animal Experiments of Biological Institute under protocol number 106/10.

AVAILABILITY OF DATA AND MATERIAL: All data generated or analyzed during this study are included in this published article.

AUTHORS' CONTRIBUTIONS: Conceptualization: Silva, A.A.; Del Fava, C. Formal analysis: Silva, A.A.; Villalobos, E.M.C.; Cunha, E.M.S.; Lara, M.C.C.S.; Nassar, A.F.C.; Piatti, R.M.; Castro, V.; Pinheiro, E.S.; Carvalho, A.F.; Del Fava, C. Funding acquisition: Del Fava, C. Investigation: Silva, A.A.; Villalobos, E.M.C.; Cunha, E.M.S.; Lara, M.C.C.S.; Nassar, A.F.C.; Piatti, R.M.; Pinheiro, E.S.; Carvalho, A.F.; Del Fava, C. Methodology: Silva, A.A.; Villalobos, E.M.C.; Cunha, E.M.S.; Lara, M.C.C.S.; Nassar, A.F.C.; Piatti, R.M.; V.; Pinheiro, E.S.; Carvalho, A.F.; Del Fava, C. Project administration: Del Fava, C. Writing – original draft: Silva, A.A.; Del Fava, C. Writing – review & editing: Del Fava, C.

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