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Causes of abortion, stillbirth, and perinatal mortality in horses[1]

Gregory D. Juffo[2], Nadia A.B. Antoniassi[3], Daniele M. Bassuino[2], Danilo C. Gomes[4], Gustavo G.M. Snel[2], Saulo P. Pavarini[2] and David Driemeier[2]*

ABSTRACT.- Juffo G.D., Antoniassi N.A.B., Bassuino D.M., Gomes D.C., Snel G.G.M., Pavarini S.P. & Driemeier D. 2022. **Causes of abortion, stillbirth and perinatal mortality in horses**. *Pesquisa Veterinária Brasileira 42:e06808, 2022.* Setor de Patologia Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil. E-mail: davetpat@ufrgs.br

Causes of abortion, stillbirth, and perinatal mortality in horses were investigated in the Department of Veterinary Pathology of the Federal University of Rio Grande do Sul (SPV-UFRGS) from 2000 to 2015. In this period, 107 cases were analyzed using macroscopic, microscopic, and complementary tests. Of these, 77 were aborted fetuses, 16 were stillbirths, and 14 were perinatal deaths. Conclusive diagnosis was established in 42.8% of the fetuses analyzed, with 28.6% classified as infectious origin, 9.1% as non-infectious, and 5.1% as other. Bacterial infections, especially those related to *Streptococcus* spp. were the most frequently observed. In stillborn foals, diagnosis was established in 62.5% of cases, and 50% of these were related to non-infectious causes, such as dystocia and birth traumas. As for perinatal mortality, a conclusive diagnosis was reached in 78.57% of cases, and infectious causes associated with bacterial infections accounted for 64.1% of these diagnoses.

INDEX TERMS: Abortion, stillbirth, perinatal mortality, horse, reproductive losses, infectious causes.

RESUMO.- [Causas de aborto, natimortalidade e morte perinatal em equinos.] Causas de aborto, natimortalidade e mortalidade perinatal em equinos foram investigadas no Setor de Patologia Veterinária da Universidade Federal do Rio Grande do Sul (SPV-UFRGS) durante o período de 2000 a 2015. Nesse período, foram analisados 107 casos através de exames macroscópico, microscópico e exames complementares, desses 77 correspondiam a fetos abortados, 16 natimortos e 14 mortes perinatais. Diagnóstico conclusivo foi estabelecido em 42,8% dos fetos analisados e classificados como origem infecciosa em 28,6% dos casos, não infecciosa com 9,1% e outros com 5,1% dos casos. As infecções bacterianas, em especial as relacionadas a *Streptococcus* spp. foram as mais frequentemente observadas. Em potros natimortos, diagnostico foi estabelecido em 62,5% dos casos, e destes, 50% foram relacionados a causas não infecciosas, como distocia e traumas durante o parto. Quanto a mortalidade perinatal, em 78,57% dos casos houve um diagnostico conclusivo, e as causas infecciosas associadas a infecções bacterianas corresponderam a 64,1% desses diagnósticos.

TERMOS DE INDEXAÇAO: Aborto, natimortalidade, mortalidade perinatal, equinos, perda reprodutiva, causas infecciosas.

INTRODUCTION

One of the main factors resulting in economic losses for horse breeders is the failure to produce a live, healthy offspring. This can be due to infertility, embryonic loss, abortion, stillbirth, and perinatal death (Giles et al. 1993, Laugier et al. 2011). Therefore, monitoring and surveillance of the causes of progeny loss over time is very important (Marenzoni et al. 2012).

The causes of abortion, stillbirth, and perinatal mortality in horses may vary over time and according to the geographical area where they are studied (Hong et al. 1993b). The knowledge of the main causes of these reproductive losses is

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[2] Setor de Patologia Veterinária, Departamento de Patologia Clínica Veterinária, Faculdade de Medicina Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil. *Corresponding author: davetpat@ufrgs.br

[3] Laboratório de Patologia Animal, Instituto de Ciências da Saúde, Universidade Federal de Mato Grosso (UFMT), Av. Alexandre Ferronato 1200, Setor Industrial, Sinop, MT 78557-267, Brazil.

[4] Laboratório de Anatomia Patológica, Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul (UFMS), Av. Senador Filinto Muller 2443, Campo Grande, MS 79074-460, Brazil.

very important to elaborate prophylactic measures to avoid the losses as much as possible (Schlafer 2004, Marenzoni et al. 2012). This study describes the main causes of abortion, stillbirth, and perinatal death in horses analyzed at the "Setor de Patologia Veterinária", "Departamento de Patologia Clínica Veterinária" of the "Universidade Federal do Rio Grande do Sul" (SPV-UFRGS) from 2000 to 2015.

MATERIALS AND METHODS

A retrospective study was conducted using SPV-UFRGS files from 2000 to 2015. The inclusion criteria were based on Platt (1973), Acland (1987), and Giles et al. (1993), who defined abortion as fetal death between 40 and 300 days of pregnancy, stillbirth as death after 300 days of pregnancy, and perinatal death as cases in which death occurs up to two weeks after delivery. The analyzed materials originated from whole, refrigerated, or frozen horses. In some cases, only tissue fragments refrigerated, frozen, or fixed in 10% formalin, were sent for analysis. Placenta was sent in 35% of the cases analyzed.

The necropsy examination of fetuses included the estimation of fetal age by measuring the sacro-occipital distance (Ginther 1992, Franciolli et al. 2011) and the evaluation of the fetus, umbilical cord, and fetal membranes (when present). In case of stillbirths or perinatal deaths, information regarding the age of the animal was provided by the owner or veterinarian in charge. In these cases, signs of dystocia and trauma were observed during the necropsy, besides possible veterinary interventions such as surgical procedures and resuscitation attempts. Tissue fragments were collected and fixed in 10% buffered formalin, routinely processed, and stained with hematoxylin and eosin (HE) for histopathological analysis (Prophet et al. 1992). Special stains, such as Gram, Grocott methenamine silver, Warthin-Starry, and Periodic Acid-Schiff (PAS), were performed when bacterial or mycotic infection was suspected, based on characteristic inflammatory changes.

Bacterial culture in ovine blood agar (5%) was performed in all cases in which necropsy was performed at SPV-UFRGS, using liver and lung fragments, stomach content, and placenta (when present). In cases suspected to have fungal involvement, lung, liver, and skin were submitted for mycological culture in Sabouraud agar. In all cases analyzed, direct immunofluorescence (DIF) was performed for *Leptospira* spp. in kidney imprints with multivalent commercial antibody at dilution of 1:20 (Miller et al. 1989). Also, immunohistochemistry (IHC) for equine herpesvirus type 1 was performed in all cases on lung and liver fragments using monoclonal antibody equine herpesvirus type 1 (HVE-1) (WMRD Pullman) at dilution of 1:100 in PBS (phosphate buffered saline). Antigen retrieval was performed with protease XVI at 0.05% for 15 min at 37°C. The primary antibody was applied for 45 min at 37°C, followed by the biotinylated secondary antibody and streptavidin solution conjugated to an alkaline phosphatase molecule (LSAB + System AP, Dako Cytomation) for 20 min each at room temperature. Permanent Red (Permanent Red/code 0695, Dako) was used as chromogen, and positive controls were inserted simultaneously with the slides tested.

Cases with conclusive diagnosis were classified by etiology as infectious and non-infectious causes. A third group called others included congenital malformations and maternal diseases not related to the reproductive system, due to the complex determination of whether infectious agents were involved or not.

RESULTS

From 2000 to 2015, 107 cases were analyzed. Of these, 77 were aborted fetuses, 16 were stillbirths, and 14 were perinatal deaths. As for the origin, 57% of animals were from the State of Rio Grande do Sul, 6.54% from Paraná, and 6.54% from Minas Gerais. Santa Catarina, São Paulo, and Alagoas each were the states of origin for 2.8% of the cases. Bahia was the origin of 1.86% of the cases, and Goiás and Maranhão each of 0.93% of the cases. In the remaining cases (17.80%), information about the origin of the animals was not available. Breed was reported in 70.96% of the animals, being distributed as follows: Quarter horse (24.29%), Criollo (13.08%), Thoroughbred (9.34%), Mangalarga Marchador (7.47%), Brasileiro de Hipismo (7.47%), Campolina (2.8%), Holsteiner (1.86%), and Appaloosa, Arabian, Lusitano, Mine horse, and Belgian Saddlebred each with 0.93% of the total. In 29.04% of cases, information about the breed was not available.

The gestational age of the aborted fetuses was established in 88.31% (68/77) of the cases. Of these, 11.69% (9/77) corresponded to the first third, 38.96% (30/77) to the second, and 37.66% (29/77) to the third gestational third. In cases of abortion, the diagnosis was established in 42.8% (33/77) of the fetuses and were classified by etiology into infectious, with 28.6% of the cases, non-infectious, with 9.1%, and others, with 5.1% of the cases. The bacteria associated with the infectious cases were *Streptococcus* spp., *Leptospira* spp., *Pasteurella pneumotropica*, *Staphylococcus* sp., and *Escherichia coli,* while *Mucor* sp. was the only agent associated with mycotic infection. In seven cases, the diagnosis was infectious abortion without defined agent. In these cases, lesions characterized by suppurative inflammatory infiltrate were observed in organs such as the placenta and lung, but it was not possible to determine the agents associated with the lesions because the refrigerated material was not sent for bacteriological culture. Non-infectious processes included umbilical cord torsion and placental insufficiency. Cases classified as others corresponded to malformation characterized by hydronephrosis and, in three cases, abortion was associated with clinical disease of the mare during pregnancy (Table 1).

Diagnoses related to bacterial infections in cases of abortion were established based on the observation of macroscopic and histological lesions associated with bacterial growth in pure culture on microbiological examination. The macroscopic lesions observed in cases of *Streptococcus* spp. infection were multifocal reddish areas in the placenta (Fig.1) (3/6). Histologically, the placenta presented with polymorphonuclear inflammatory infiltrate, sometimes pyogranulomatous, with bacterial colonies (4/6), and the lung had inflammatory infiltration of neutrophils, sometimes mononuclear cells, and coco-bacillary bacterial clumps within alveoli associated with fibrin deposition (5/6). The diagnosis of abortion by *Leptospira* spp. was based on the observation of fetal jaundice associated with areas of diffuse hemorrhage in the skin, mucous membranes, and organs, histologically correlated with mononuclear interstitial nephritis and severe vasculitic changes in the placenta. The cases were confirmed by DIF on kidney imprints. In one of the cases, spirochetes compatible with *Leptospira* spp. were observed in the kidney with Warthin-Starry staining.

The cases of abortion by *P. pneumotropica* presented macroscopic changes in the placenta, with congested

blood vessels (1/2) and extensive thickened areas with hemorrhage (1/2). Microscopically, multiple basophilic bacterial colonies were diffusely distributed in the tissue, associated with polymorphonuclear inflammatory infiltrate (2/2). In abortion due to *E. coli* infection, macroscopic examination showed focal area of hemorrhage in cerebral cortex, mild jaundice in subcutaneous tissue, hepatomegaly, and hydrothorax. Microscopic analysis showed multifocal suppurative bronchopneumonia and hepatitis associated with bacterial aggregates, besides bacterial thrombi in the vessels of these organs and in meninges. In the case in which abortion was associated with *Staphylococcus* sp. infection, the fetus presented no macroscopic changes; the only changes observed were microscopically in the placenta, with mild suppurative inflammation and multiple bacterial aggregates.

In the case related to mycotic infection, macroscopic examination showed moderate cotyledon thickening (Fig.2), which histologically corresponded to marked necro-suppurative placentitis associated with fungal structures. Suppurative pneumonia was histologically observed in the fetus. The diagnosis was confirmed by Grocott methenamine silver stain with observation of fungal structures in the placenta and isolation of *Mucor* spp. in mycological culture.

The diagnosis of non-infectious causes was established by fetal and umbilical cord macroscopic analysis. In placental insufficiency, the fetus presented low body development compared to the one expected for the gestational age and also moderate to marked fatty liver degeneration. Umbilical cord changes were characterized by torsion greater than 360° with increased volume and umbilical congestion. Cases in which the etiology of the loss was related to maternal diseases were based on the absence of macroscopic and histological changes in the fetus associated with clinical data and anamnesis of the mother compatible with this outcome. This condition occurred in three situations. In the first, the mare presented with spasmodic colic; in the second, it was due to a clinical condition of tetanus; and in the third, it was associated with maternal septicemia. The congenital change observed in one fetus was characterized by hydronephrosis.

Of the 16 stillborn foals analyzed, 62.5% (10/16) of the cases were diagnosed (Table 2). Of this total, one case was associated with an infectious agent with isolation of *Streptococcus* spp., and eight were associated with non-infectious causes such as dystocia, birth trauma, placental insufficiency, and asphyxia. Additionally, there was one case of congenital malformation characterized by arthrogryposis. Cases of dystocia were characterized by the presence of petechiae and suffusions on the mucous membranes of the stillborn, as well as congestion in the fetal organs with no histological lesions caused by infectious agents associated with the anamnesis. Cases of trauma during delivery were diagnosed through history and observed lesions; in both cases, there was a large amount of blood in the abdominal cavity with formation of clots adhering to the omentum, liver laceration, and absence of histological changes.

The cause of perinatal death in foals was established in 78.57% (11/14) of cases (Table 3). The associated infectious agents were *Actinobacillus equuli*, *Staphylococcus* spp., *Streptococcus* spp., *Klebsiella pneumoniae*, and *Enterobacter aerogenes*. In two cases, inflammatory lesions, suppurative meningoencephalitis and bronchopneumonia were observed, but the agent was not determined. There were two cases of

Table 1. Causes of abortion diagnosed in horses from 2000 to 2015

Causes of abortion	Cases	% of total
Infectious		
Bacterial		
Streptococcus spp.	6	7.8
Leptospira spp.	4	5.2
Pasteurella pneumotropica	2	2,6
Staphylococcus spp.	1	1.3
Escherichia coli	1	1.3
Fungal		
Mucor spp.	1	1.3
No defined agent	7	9.1
No infectious		
Umbilical torsion	4	5.2
Placental insufficiency	3	3.9
Others		
Congenital abnormalities	1	1.3
Maternal diseases	3	3.9
Not determined	44	57.1
TOTAL	77	100

Table 2. Causes of stillbirth diagnosed in horses from 2000 to 2015

Causes of stillbirth	Cases	% of total
Infectious		
Bacterial		
Streptococcus spp.	1	6.2
Non infectious		
Dystocia	3	18.8
Placental insufficiency	2	12.5
Birth trauma	2	12.5
Asphyxia	1	6.2
Others		
Congenital abnormalities	1	6.2
Not determined	6	37.5
TOTAL	16	100

Table 3. Causes of perinatal death in horses diagnosed from 2000 to 2015

Causes of perinatal death	Cases	% of total
Infectious		
Bacterial		
Acinobacillus equuli	2	14.2
Streptococcus spp.	1	7.1
Klebsiella pneumoniae	1	7.1
Enterobacter aerogenes	1	7.1
Staphylococcus spp.	1	7.1
No defined agent	3	21.5
Others		
Congenital abnormalities	2	14.2
Not determined	3	21.5
TOTAL	14	100

congenital malformation, one characterized by arthrogryposis and the other by congenital inguinal hernia with consequent peritonitis. *A. equuli* infection was related to cases of septicemia in 1-day-old foals. Macroscopically, there was a renal lesion characterized by whitish nodules with a diameter of approximately 0.2cm on the capsular surface, which were more evident in the cortical region after cutting. Histologically, it corresponded to marked multifocal to coalescent inflammatory infiltrate with predominance of neutrophils, associated with basophilic areas of slightly granular aspect. In the case of *Staphylococcus* sp. infection, the foal presented with increased volume in the left pelvic limb with marked subcutaneous edema and a large amount of thick, turbid, reddish material in the joint cavity of the femur-tibia-patella joint. There were also purulent exudate collections between the parietal and visceral pleura, and the lungs presented multifocal areas of consolidation associated with hemorrhage (Fig.3). Histological examination showed multifocal suppurative inflammatory infiltrate with fibrin, besides hemorrhagic infarcts and purulent fibrinous pleuritis in the lungs. The case associated with *Streptococcus* sp. in the right pelvic limb macroscopically showed edema in the subcutaneous tissue and purulent secretion between the muscles. The femur-tibia-patella joints of the right and left limbs and the humerus-radius-ulna joint of the left limb presented with synovial fluid with increased volume, yellow color, and fibrin deposition. Histological examination of the joints showed synovial proliferation with marked neutrophil infiltration in the joint capsule and lumen, inguinal lymph node with marked neutrophil and macrophage infiltration, and moderate diffuse congestion in the lung.

The case related to *K. pneumoniae* macroscopically presented with fibrinous exudate on the pleural and pericardial surface and reddish lungs with firm areas (Fig.4). The small intestine presented with an area of intussusception. Histological examination showed moderate multifocal mixed pneumonia, hemorrhage, and marked edema, besides fibrinous pleuritis and pericarditis associated with bacterial aggregates. The case of *E. aerogenes* infection showed fibrin deposition in the meninges on the encephalon, multifocal dark spots, besides diffuse congestion and edema in the lungs and purulent exudate in the joints. Histological examination revealed meningoencephalitis and marked purulent myelitis with associated Gram-negative bacteria, suppurative bronchopneumonia with bacterial emboli in the vessels, and suppurative arthritis.

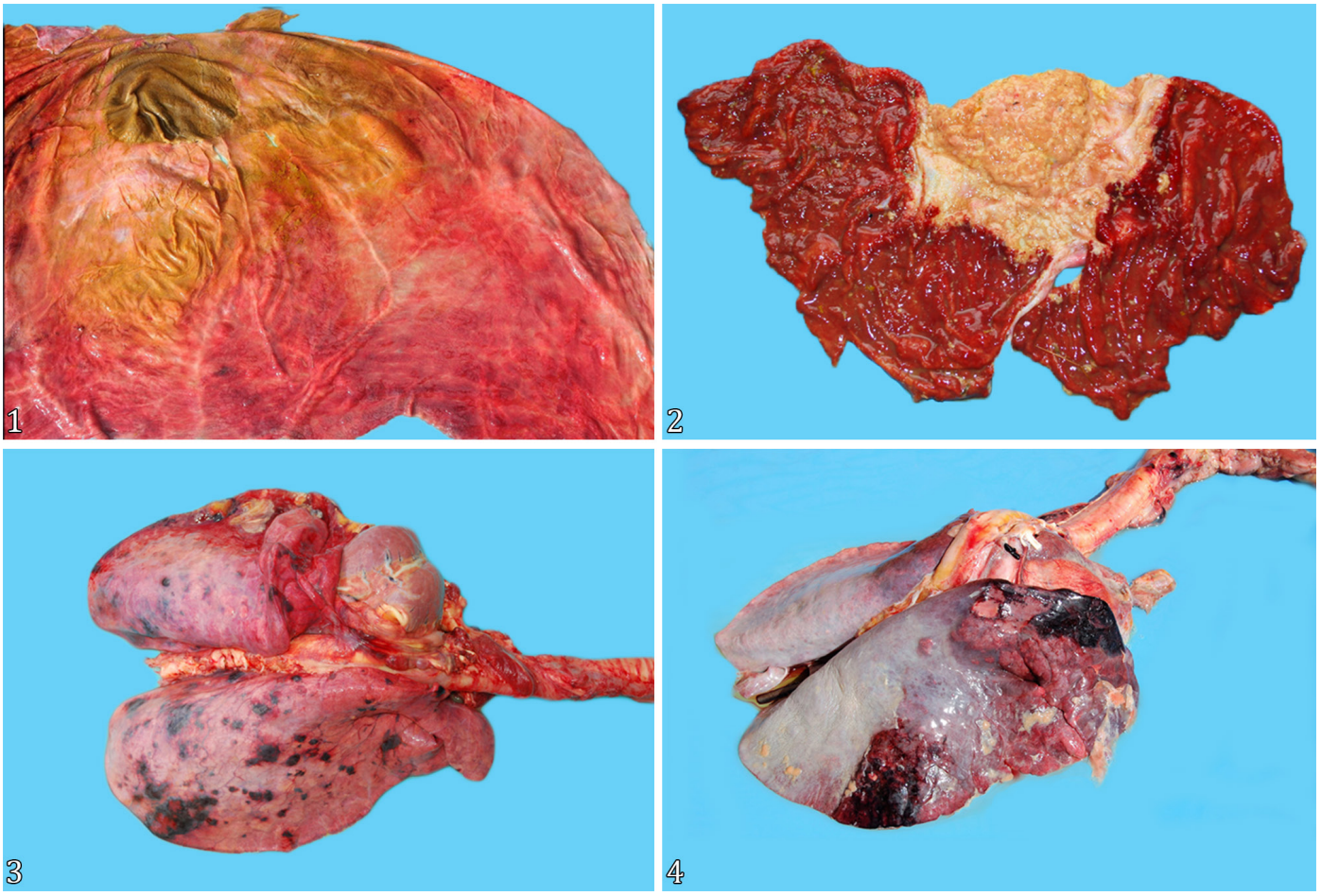

Fig.1-4. Causes of abortion, stillbirth, and perinatal mortality in horses. (**1**) Abortion by *Streptococcus* sp. Placenta with reddish multifocal areas. (**2**) Abortion by *Mucor* sp. Placenta with marked thickening of cotyledons and intense red coloration. (**3**) Perinatal death due to *Staphylococcus* sp. Lungs with dark red multifocal areas that corresponded to areas of consolidation and hemorrhage. (**4**) Perinatal death due to *Klebsiella pneumoniae*. Lung with areas of cranioventral consolidation, focally extensive areas of dark red color, and fibrin deposition on the pleural and pericardial surface.

DISCUSSION

In this study, it was possible to establish the cause of abortion in 42.8% of cases, stillbirth in 62.5%, and perinatal mortality in 78.57% of the cases analyzed. This percentage is lower than those reported by Tengelsen et al. (1997) and Hong et al. (1993b), with 67.3% and 83.1% of conclusive cases, respectively. A possible explanation for this result would be the inadequate use of samples, for example, the low use of placenta in cases of abortion, and the lack of detailed history in most of the cases analyzed. Diagnosis is possible in most cases when the fetus and especially the placenta are sent to the laboratory (Smith et al. 2003, Marcolongo-Pereira et al. 2012). In this study, the cases of abortion occurred mainly in fetuses older than six months. Abortions occurring earlier are probably underestimated in this type of study, as it is usually difficult to find these smaller fetuses (Smith et al. 2003, Laugier et al. 2011). Also, there is possibly less interest from owners in trying to discover the possible causes of these losses. The predominance of horses with defined breed, 70.96%, suggests that sending the materials would be related to the greater economic value of these horses, as well as the interest in avoiding new cases.

During the analyzed period, infectious causes were more frequent in cases of abortions and perinatal death. This result corroborates studies in France (Laugier et al. 2011) and in Brazil (Marcolongo-Pereira et al. 2012), being different from those in the USA (Hong et al. 1993b, Tengelsen et al. 1997) and in the UK (Rickets et al. 2003, Smith et al. 2003). There are varied causes of abortion in horses due to regional differences associated with husbandry practices, geographic area, and characteristics of the equine population studied (Hong et al. 1993a).

Infectious causes of bacterial origin were predominant in cases of abortions and perinatal mortality. This predominance was also found in the studies by Giles et al. (1993), Laugier et al. (2011), and Marcolongo-Pereira (2012), which accounted for 53.1%, 79.9%, and 68.4% of cases, respectively. In cases of *Streptococcus* spp. infection, the lung was the most important organ for isolation of the agent and observation of histological lesions, which occurred in most cases in this organ. *Streptococcus* spp. is ubiquitous in horse husbandry, so bacterial isolation should be cautiously interpreted when they are isolated at the end of pregnancy. The criteria for the diagnosis of *Streptococcus* are the isolation of this bacterium from fetal organs and compatible histological changes (Hong et al. 1993a). Lymphadenitis and polyarthritis were observed in the case of perinatal death, in which this agent was also isolated, a report similar to that described in pigs with this condition associated with *Streptococcus* spp. infection (Kawata et al. 2003). Due to the wide dissemination of histological lesions, the infection is believed to have occurred at birth, and septicemia resulted in cardiorespiratory arrest.

The result obtained in this study indicates the important role of *Leptospira* sp. in cases of abortions, as observed by other authors (Hodgin et al. 1989, Gilles et al. 1993, Poonacha et al. 1993, Marcolongo-Pereira 2012). The diagnosis was based on macroscopic and microscopic lesions similar to those described by Hodgin et al. (1989) and Poonacha et al. (1993), and confirmed by DIF on kidney sections. The other bacteria associated with the cases occurred in isolation within the herd. In addition, they are not frequently associated with causes of reproductive losses in horses. These opportunistic bacteria, commonly commensal, may cause sporadic abortions in production animals (Ward et al. 1998, Corbellini et al. 2006). A possible route of infection is transplacental after uterine contamination, causing abortions and deaths by septicemia soon after birth (Schlafer & Miller 2007).

It was not possible to identify a pathogen in ten cases with histological lesions in the placenta and/or lung because those cases had only formalin-fixed material, which made microbiological isolation unfeasible. However, these lesions were similar to those observed in cases associated with identified infectious agents.

Fungal placentitis caused by *Mucor* spp. was confirmed by histological changes and the presence of hyphae morphologically compatible with that of the genus and isolation of the agent from the placenta. Due to the great dissemination of fungi in nature, only the isolation of the agent in cases of abortion is not enough for a conclusive diagnosis, with the observation of the usual morphology of the fungus in histopathology being necessary (Driemeier et al. 1998). Equine placentitis associated with the genus *Mucor* sp. had been described (Giles et al. 1993, Tengelsen et al. 1997); however, fetal invasion is rare (Whitwell 1980).

Some conditions that lead to abortion, such as herpesviruses and twin pregnancy, have a lower occurrence in some areas (Schlafer 2004), as observed in this study. Despite performing IHC to detect HVE-1 in fetal tissues in all cases, no positive result was observed. The low frequency of viral abortion in horses in southern Brazil was reported by Marcolongo-Pereira et al. (2012) and Estima-Silva et al. (2019), being possibly associated with the use of efficient vaccines (Schlafer 2004, Marcolongo-Pereira et al. 2012).

Of the non-infectious causes, abortion due to umbilical torsion is attributed to vascular damage in the umbilical cord due to excessive torsion, edema, hemorrhage, and/or thrombosis of umbilical vessels, a disorder reported in other studies (Rickets et al. 2003, Szeredi et al. 2008). Placental insufficiency results in abortion due to a delay in placental development, which compromises the passage of nutrients and consequently fetal growth, being usually associated with pre-existing endometriosis and fibrosis (Schlafer 2004). This condition is seen mainly in older mares (Swerczek 1991). However, this correlation with the age of the mares could not be established in the three cases, as there was no access to these data. Similar to what was demonstrated by Antoniassi et al. (2013) in cases of bovine abortion, it is believed that non-infectious causes are underestimated, since there is great difficulty in diagnosis due to the absence of histological lesions and the need for a detailed history of the animal and farm.

Cases of stillbirths associated with dystocia and perinatal asphyxia were based on macroscopic changes and history. This condition may result from any element delaying the expulsion of the fetus (Hong et al. 1993a). Cases of birth trauma can occur in unassisted deliveries and, as described by Swerczek (1990), trauma to the chest wall, heart, and other internal organs can be significant factors in death during delivery.

Congenital abnormalities identified accounted for 4.8% of the cases, a value similar to the one reported by other authors (Whitwell 1980, Smith et al. 2003). In the three cases of abortion associated with maternal diseases, the cause of fetal loss was related to physiological changes caused by the release of substances by the mother. As observed by Swerczek (1991), stress due to the environment, diseases, or medication in late pregnancy increases the risk of gestational loss.

CONCLUSIONS

The diagnosis was established in 50.4% of all the material analyzed, with a greater portion related to infectious causes of bacterial origin. There was a higher percentage of diagnosis in cases of stillbirth and perinatal death, corroborating the literature, reflecting the great challenge in the elucidation of the causes of abortion. It was also evidenced that sending the placenta for analysis increases the possibility of conclusive diagnosis.

The improvement of diagnostic techniques, as well as the strengthening of the relationship with veterinary clinicians, is necessary to improve the efficiency of diagnosis and thus reduce the occurrence of reproductive losses.

Conflict of interest statement.- The authors declare that they have no competing interest.

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