Bacterial, protozoal and viral abortions in sheep and goats in South America: A review

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Highlights

- Bacterial/protozoal/viral abortifacients of South American sheep/goats are reviewed.
- Confirmed, probable or possible causes of abortion are categorized.
- Salient fetoplacental lesions and diagnostic laboratory tests are summarized.
- Small ruminant abortifacients never reported in South America are listed.

Abstract

Bacterial, protozoal and viral diseases are major causes of abortion in sheep and goats. These agents cause significant economic losses, and many are considered of concern for public health (zoonotic pathogens) and/or the international trade of livestock, such as those causing diseases notifiable to the World Organization of Animal Health (OIE). In South America, information about their occurrence, prevalence and economic impact is scarce. We review the available literature on bacterial, protozoal and viral abortifacients identified through laboratory testing in sheep and goats in South America and discuss whether the diagnostic investigations are conclusive in demonstrating abortion causality. We also compile information on diagnostic methods recommended by the OIE for the laboratory diagnosis of these abortifacients and on salient fetoplacental lesions induced by them. Campylobacteriosis (*Campylobacter fetus* subsp. *fetus*), listeriosis (*Listeria ivanovii*), chlamydiosis (*Chlamydia abortus*), toxoplasmosis, neosporosis and sarcocystiosis have been confirmed as small ruminant abortifacients in this region. *Brucella ovis*, *Brucella melitensis*, *Campylobacter jejuni*, *Chlamydia pecorum*, *Coxiella burnetii*, *Leptospira* spp., *Bacillus licheniformis* and bluetongue virus, are probable causes of abortion in the region since they have been detected in aborted fetuses and/or associated with abortions through seroepidemiologic studies. *Listeria monocytogenes*, *Histophilus ovis*, *Actinobacillus seminis*, *Trueperella pyogenes*, *Yersinia* spp., *Trypanosoma vivax*, caprine herpesvirus 1 and pestiviruses also infect small

ruminants in the region and could thus be considered possible causes of abortion, although they have not been associated with abortion in South America (i.e., not detected in aborted fetuses nor associated with abortion through seroepidemiologic studies). Other agents such as *Flexispira rappini*, *Francisella tularensis*, *Anaplasma phagocytophilum*, Rift Valley fever virus, Wesselbron disease virus and bunyaviruses, known to be abortifacients for sheep and goats in other regions of the world, have not been documented in South America. While some of these agents could be exotic in this subcontinent, others may have been undiagnosed considering the limitations of active animal disease surveillance systems, which hamper the eventual detection of emerging, re-emerging, and communicable diseases in South America.

Keywords: abortions, diagnostic investigation, goats, infectious diseases, pathology, protozoa, sheep, South America.

1. Introduction

Sheep and goats were introduced in South America by Spanish and Portuguese colonizers during the 16th century. Currently, sheep and goat farming are economically and culturally important activities in South America. This region harbors approximately 65 million sheep and 22 million goats, accounting for 5% and 2% of the global population, respectively (FAO, 2018).

Small ruminant husbandry is often considered a secondary agricultural activity and is used as a means of subsistence in many small family farms (Aréchiga et al., 2008). Due to their high adaptability to hostile environments, sheep and goats are usually raised on marginal lands, unsuitable for more profitable agricultural activities. Rearing is carried out in varying farming systems, including extensive, intensive, nomadic and/or transhumant conditions (Aréchiga et al., 2008; Smith and Sherman, 2009; Zygoyiannis, 2006).

In South America, small ruminant production levels are generally low due to multiple factors, including, but not limited to, nutritional, sanitary and reproductive problems (Alexandre and Mandonnet, 2005; FAO, 2018; Morris, 2009). Reproductive failure, including abortion, directly influences the number of weaned lambs/kids, culling percentage and replacements (Bonino Morlan, 2004), causing significant economic losses by decreasing the overall flock/herd productivity (Edmondson et al., 2012). Sheep and goats usually have a higher incidence of abortion than cattle. Abortion rates up to 5% are often considered normal (Edmondson et al., 2012). Bacterial, parasitic (protozoal), viral, nutritional, genetic, metabolic and toxic causes of abortion have been identified (Borel et al., 2014). Among them, bacterial, protozoal and viral diseases are the most frequently recognized causes of abortion in sheep and goats worldwide (Kirkbride, 1993; Moeller, 2001).

The importance of studying causality of abortion in small ruminants relies on six main reasons: 1- many bacterial, viral and protozoal abortifacient pathogens are zoonotic, 2- some

of them cause diseases that are notifiable to the World Organization of Animal Health (OIE) since they imply specific hazards for the international trade of livestock or animal products (OIE, 2020), 3- they negatively impact on the environment by reducing animal productivity resulting in increased greenhouse gas emissions per unit of livestock product (Hristov et al., 2013), 4- they affect the economy of subsistence farmers in deprived areas contributing to poverty and food insecurity, 5- the recent emergence of bacterial abortifacients resistant to antibiotics (Sahin et al., 2008), and 6- reproductive losses can be a negative indicator of animal welfare.

Despite these reasons, bacterial, protozoal and viral causes of abortion in small ruminants have not been systematically studied in most South American countries (Menzies, 2011). While some of these pathogens have long been recognized by multiple diagnostic tests, others have been identified only through indirect methods (serology). Many of these abortifacients have not been investigated in South America or are considered exotic. Regional publications on this topic are scarce and often difficult to access. The available reviews focus on a single disease in a country (da Silva et al., 2013; Poester et al., 2002; Rovani Scolari et al., 2011; Samartino, 2002) or region (Jones and Dávila, 2001; Lager, 2004; Legisa et al., 2014; Lobato et al., 2015; Martins and Lilenbaum, 2014; Petrakovsky et al., 2014; Rossetti et al., 2017), but no comprehensive reviews on a variety of bacterial, protozoal and viral abortifacients are currently available.

The etiologic diagnosis of abortion in ruminants is often challenging due to the difficulty in acquiring quality samples under extensive field conditions, poor record keeping of health events at the farm level, the lack of access to specialized animal health laboratories with validated diagnostic tests, and the limited understanding of the clinical and pathological manifestations caused by abortifacients. This work reviews the available literature on bacterial, protozoal and viral causes of abortion identified in sheep and goats in South

America through laboratory testing. We discuss whether the diagnostic investigations presented in the revised literature are conclusive in demonstrating abortion causality, as well as their differential diagnoses. Lastly, we compile information on diagnostic methods recommended by the OIE to assess causes of abortion in small ruminants, as well as on salient fetoplacental lesions for each agent and the gestational period of the abortions induced by these agents.

2. Materials and methods

A literature search was conducted using several databases (Google Scholar, SciELO, Latindex, ScienceDirect, MEDLINE, WorldWideScience and BASE). Search terms included combinations of sheep, goat, ovine, caprine, names of all South American countries (Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Perú, Suriname, Uruguay and Venezuela), current or former Latin names of bacterial, protozoal and viral causes of abortion (i.e. *Toxoplasma gondii*, *Chlamydia abortus*, *Chlamydophila abortus*, *Brucella* spp., *Coxiella burnetii*, bluetongue virus, *Orbivirus*, etc.), names of diseases (i.e. toxoplasmosis, chlamydiosis, ovine enzootic abortion, brucellosis, coxiellosis, Q fever, bluetongue disease, etc.), and the term abortion. There were no restrictions regarding the type of study, year of publication (last search performed in March 2021), or language (Spanish, Portuguese, English and French). Peer-reviewed scientific papers (112 documents), conference abstracts (20 documents), bachelors (three documents), masters (one document) and PhD (two documents) theses from all South American countries were similarly considered. Additionally, one book (Robles and Olaechea, 2001) and three trustable non-peer reviewed publications including two official bulletins from national animal health authorities from Chile (Saldías et al., 2014) and Perú (Valderrama et al., 2015), and an OIE performance of veterinary services evaluation report for Uruguay (Hutter et al., 2014)

were included. To enrich the discussion and regardless of their geographical origin, we consulted other 44 peer-reviewed scientific papers, one peer-reviewed conference abstract, eight textbooks and two websites, including the statistics of the Food and Agriculture Organization of the United Nations (FAOSTAT) (FAO, 2018) and the OIE listed diseases, infections and infestations in force in 2020 (OIE, 2020).

Based on the compiled information, we deliberately classified the diseases/agents identified in South America in one of three categories: 1- *confirmed*, 2- *probable*, or 3- *possible cause of abortion*. Category 1- *confirmed cause of abortion* included abortifacients that were detected in the placenta and/or fetal samples of aborted sheep/goats, coupled with identification of compatible gross and/or histologic lesions along with exclusion of other abortifacients known to cause similar lesions. Category 2- *probable cause of abortion* included: a- abortifacients that were identified in the placenta and/or fetal samples of aborted sheep/goats without describing compatible pathology, or with compatible pathology for which other abortifacients that can cause similar lesions (differential diagnoses) where not ruled out; and b- agents that were investigated through seroepidemiologic studies for which a statistical association between abortion and seropositivity was found. Category 3- *possible cause of abortion* included agents that, even though they are known for their abortigenic potential and are present in sheep and/or goats in South America as demonstrated either through direct or indirect laboratory methods, they have not been identified in cases of abortion in sheep/goats in the region.

3. Abortifacients in South America

Bacterial, protozoal and viral abortifacients identified in sheep and/or goats in South America and their categorization as confirmed, probable or possible causes of abortion as defined above are summarized in Table 1. In this table, pathogens detected in only one

livestock species in a given country, were considered as a possible cause of abortion for the other species in the same country. Samples and diagnostic methods recommended by the OIE to assess causes of abortion in small ruminants, as well as salient fetoplacental lesions and the gestational period of the abortions induced by these agents are shown in Table 2.

3.1. Bacteria

3.1.1. Brucella ovis

Brucella ovis is transmitted venereally, and causes ovine epididymitis/orchitis with reduced fertility, occasional abortions and perinatal mortality (Moeller, 2012). In South America, *B. ovis* was first isolated from affected rams in the 1960s in Argentina (Cedro et al., 1963). Years later, brucellosis was diagnosed in Rio Grande do Sul, Brazil (Blobel et al., 1972), where nine isolates of what the authors called *Brucella*-like bacteria (morphologically and biochemically consistent with *B. ovis*) were cultured from the epididymis or semen of 9/25 rams with epididymitis. A tenth isolate was cultured from the stomach content of 1/17 stillborn lambs. The same authors used six of these isolates (including the one from the stillborn lamb) to experimentally reproduce epididymitis in six rams (Blobel et al., 1972).

Despite the broad distribution of *B. ovis* in South American sheep flocks (Corbel, 1997), our search did not retrieve any publications describing abortions with fetal and/or placental lesions and concurrent identification of this agent. Consultation with an expert in ovine brucellosis from the Argentinian "Instituto Nacional de Tecnología Agropecuaria" (INTA), supported this observation (Robles C., personal communication 2020). Since the disease affects rams more often than ewes, most serologic surveys have focused on the former, and only a few serologic studies attempted to associate the seropositivity with reproductive problems in ewes (Alves et al., 2017; Pinheiro Junior, 2008; Rizzo et al., 2014). In São Paulo (Brazil), a seroprevalence of 1.7% was reported using complement fixation test

(CFT) in 294 sheep from 28 flocks with history of reproductive disorders. Of the five seropositive sheep, four were ewes, three of which had a history of abortion and one of estrus repetition. The remainder seropositive animal was an asymptomatic ram. No association between seropositivity and reproductive disorders was found (Rizzo et al., 2014). In the same study, selective bacterial cultures for *Brucella* spp. on 16 aborted fetuses, one placenta, 13 uterine discharges and six vaginal swabs from aborted sheep were negative for *B. ovis* (Rizzo et al., 2014). In Pernambuco, Brazil, sera from 119 ewes from 12 farms were tested by agar gel immunodiffusion (AGID), revealing a seroprevalence of 5.88% (Alves et al., 2017). Seropositivity was significantly associated with the occurrence of abortion, since five seropositive and 28 seronegative ewes had a history of abortion, while the remainder 84 seronegative and two seropositive ewes did not (Alves et al., 2017).

Since most serologic surveys focused mainly on rams, the seroprevalence of brucellosis in ewes is largely unknown. Robles et al. (2014) surveyed 208 ewes and 147 rams from 28 farms located in Río Negro province, Argentina. The seroprevalence determined by ELISA was 4.81% and 9.52% in ewes and rams, respectively. No association between abortions and serological status was attempted (Robles et al., 2014). An extensive 15-year study in the Argentinean Patagonia including 181,495 serum samples of rams from 758 farms found a seroprevalence of 5.8% and 66.2% at the individual and farm levels, respectively (Robles et al., 2012). This indicates that the agent is widespread in the region regardless of the documentation of abortions. Of all other South American countries, there is evidence of *B. ovis* seropositivity in rams in ovine flocks in Chile (Arévalo Hernández, 2004) and Perú (Quispe et al., 2002; Véliz and Pizarro, 1997).

The pathologic hallmark in abortions caused by *B. ovis* is suppurative placentitis with cotyledonary and intercotyledonary necrosis, and small intratrophoblastic gram-negative coccobacilli (Moeller, 2012). In addition, aborted fetuses may have bronchopneumonia,

splenomegaly and fibrinous peritonitis or pleuritis (Moeller, 2012). The etiologic diagnosis is confirmed by bacterial isolation from the placenta or fetal samples in blood agar or, preferably, Thayer–Martin's medium (OIE, 2018) or Skirrow agar (Paolicchi et al., 1991). Additionally, specific anti-*B. ovis* antibody detection can be performed in maternal serum using ELISA, CFT or AGID assays (OIE, 2018). Bacterial DNA or antigen detection can be achieved in fetal tissues or placenta by PCR and immunohistochemistry (IHC), respectively (Moeller, 2012).

Based on the criteria we established, *B. ovis* was categorized as a probable cause of abortion in ewes in Brazil. In this country, the agent was isolated from a stillborn lamb not subjected to pathologic examination, and statistically associated with abortion in a seroepidemiologic study. In Argentina, Chile and Perú, *B. ovis* should be considered a possible cause of abortion as serological studies indicate exposure.

3.1.2. Brucella melitensis

Brucella melitensis infects goats, sheep and humans (Blasco and Molina-Flores, 2011). In sheep and goats, this agent causes late gestation abortion more often in goats than sheep. Caprine brucellosis has been controlled in most developed countries but remains endemic in many developing countries (Blasco and Molina-Flores, 2011; Rossetti et al., 2017). Russo et al. (2016) studied the prevalence of *B. melitensis* in goats and sheep from Formosa, Argentina. A total of 25,401 caprine and 2,453 ovine sera were tested for antibodies against smooth *Brucella* spp. by buffered plate antigen (BPA) test and CFT, revealing seroprevalences of 12.6% and 1.4% in goats and sheep, respectively. *Brucella melitensis* was detected through bacterial culture and PCR in milk samples in 5/15 goats from three different herds with a recent history of abortion (Russo et al., 2016).

The seroprevalence of *B. melitensis* has been estimated through a few serologic surveys in goats in Perú (Garro et al., 2005; Rojas et al., 2006; Taboada et al., 2005; Toledo et al., 2007; Valderrama et al., 2015) and Ecuador (Poulsen et al., 2014), and sheep and goats in Venezuela (Arias and Cárdenas, 2007; Javitt et al., 2009; Valeris-Chacín et al., 2012). Association between serologic results and abortion was not determined in any of these surveys. Goat flocks in the Argentinian Patagonia, Colombia, Chile, Uruguay and Brazil are considered free of caprine brucellosis (Poester et al., 2002; Rossetti et al., 2017; Tique et al., 2010).

A case of ovine abortion caused by *B. melitensis* was reported in an Argentinian farm where goats and sheep coexisted (Gaido et al., 2015). An ovine fetus aborted at 4.5 months of gestation exhibited grossly visible fibrinous polyserositis and hepatosplenomegaly, lesions compatible with brucellosis. Histologically, mononuclear interstitial pneumonia, perivascular splenitis and diffuse placentitis were described (Gaido et al., 2015). Despite the hepatomegaly described grossly, no histologic lesions were described in the liver, although a complete list of the examined tissues was not provided. A bronchopneumonia consisting of infiltrates of neutrophils and macrophages rather than mononuclear interstitial pneumonia would have been expected with most abortigenic bacterial diseases, including brucellosis, which can also induce the formation of multinucleated giant cells (Kirkbride, 1993; Moeller, 2012; Poester et al., 2013). Information on the predominant types of inflammatory cells infiltrating the spleen and placenta, the occurrence of necrosis in the affected tissues, as well as whether intratrophoblastic and/or extracellular intralesional coccobacilli were observed in the placenta or fetal tissues was omitted. *Brucella melitensis* was isolated and detected by PCR from the lung, liver and abomasum of the aborted fetus. *Brucella ovis* was ruled out by PCR. Agglutination with monospecific sera identified the isolate as *B. melitensis* biovar 1 (Gaido et al., 2015), which is the biovar prevailing in South America (Samartino, 2002).

Sections of placenta, spleen, liver and lung were processed by IHC using a commercial antiserum against *Brucella abortus* as the primary antibody. There was positive immunoreactivity in the placenta and lung, which was interpreted as a cross-reaction with *B. melitensis* (Gaido et al., 2015). In this case, *B. abortus* was not investigated by PCR. Serologic assessment of all the adult goats of the herd (n = 168) and some of the sheep (n = 6, including the aborted ewe) using BPA as a screening test and CFT as a confirmatory test revealed seroprevalences of 69% and 50% in the goats and the sheep, respectively, with the aborted ewe being seropositive (Gaido et al., 2015). Other causes of placentitis and abortion, including *T. gondii*, *Campylobacter* spp., *Chlamydia* spp. and *C. burnetii* seem to not have been investigated in this case.

Lesions and diagnostic procedures for *B. melitensis* are similar to those described for *B. ovis* in the previous section. Although the available serological techniques [BPA, CFT, Rose Bengal Test (RBT), etc.] were originally developed for the detection of anti-*B. abortus* antibodies in cattle, their use is also recommended for small ruminants (Blasco and Molina-Flores, 2011; OIE, 2018).

Based on our classification criterion, *B. melitensis* is a probable cause of abortion in sheep and a possible cause of abortion in goats in Argentina, and a possible cause of abortion in Ecuador, Perú and Venezuela where there is serologic evidence of exposure.

3.1.3. Campylobacter spp.

Campylobacter fetus subsp. *fetus* (*Cff*) and *C. jejuni* are major causes of abortion in sheep and goats in the USA (Kirkbride, 1993; Moeller, 2001). In South America, reproductive losses due to *Campylobacter* spp. have been seldomly reported. Fiorentino et al. (2017) described an outbreak of abortion in 7 of 205 Pampinta ewes from La Pampa, Argentina. Twin fetuses and three placentas were sampled for diagnostic investigation, *Cff*

was isolated in pure culture and/or identified by PCR from both fetuses and two placentas. Histopathologic examination revealed a suppurative bronchopneumonia and pericarditis compatible with campylobacteriosis in both fetuses.

Sporadic abortion caused by *Cff* was reported in one of 62 Finnish sheep in Uruguay (Dorsch et al., 2021; Giannitti et al., 2018). The fetus was not recovered, but the placenta of the aborted ewe had a severe fibrinosuppurative placentitis with neutrophilic and necrotizing vasculitis and thrombosis in the chorionic arterioles, which are typical of campylobacteriosis. *Campylobacter fetus* was isolated and further identified to the species and subspecies levels by multiplex and real-time PCRs (Dorsch et al., 2021) and whole genome sequencing (Costa et al., 2020). Other causes of placentitis including *T. gondii*, *Chlamydia* spp. and *C. burnetii* were ruled out by IHC (Dorsch et al., 2021). The isolated *Cff* strain showed resistance to tetracyclines, nalidixic acid, telithromycin and clindamycin, meeting the criteria to be considered a multidrug resistant strain (Dorsch et al., 2021). In Brazil, *Cff* DNA was detected by PCR in an ovine fetus aborted at 105 days of gestation and an ovine neonate from two different properties in the state of Rio Grande do Sul. In this case, no histologic lesions were identified, the agent was not isolated on microaerobic cultures in either case, and other common causes of abortion and neonatal mortality, including *T. gondii* and *Chlamydia* spp., were not ruled out (Gressler et al., 2012). In this context, the causality of abortion or neonatal mortality is questionable, as confirmation of the etiologic diagnosis should not be based on molecular detection alone.

The importance of *C. jejuni* as an abortifacient in other regions of the world seems to be higher in goats than sheep (Smith and Sherman, 2009). However, there are no reports of abortions caused by *C. jejuni* in goats in South America. In Rio Grande do Sul (Brazil), this bacterium was isolated in pure culture and identified by PCR from the abomasal content of an aborted ovine fetus, which is highly suggestive of causality. No lesions were reported at

necropsy and no results of histopathologic examination of the fetal tissues were provided (Vargas et al., 2005). In São Paulo (Brazil), *C. jejuni* was isolated from the feces of 7 of 274 sheep from 5 flocks with history of reproductive disorders, including the birth of weak lambs, neonatal death and retained placentas (Rizzo et al., 2015). To date, we have not found publications describing fetal or placental lesions in aborted ovine fetuses infected with *C. jejuni* in South America. Although as the agent infects sheep flocks in the region, underreporting of abortion by *C. jejuni* is likely.

A *Campylobacter* sp. was detected by PCR in an aborted ovine fetus in a backyard flock of 23 sheep in Uruguay (Aráoz et al., 2018). At necropsy, the fetus had fibrinous polyserositis accompanied by moderate hydroperitoneum, hydrothorax and hydropericardium. Histologic examination revealed multifocal random necrotizing and neutrophilic hepatitis, multifocal neutrophilic pneumonia (alveolitis), and moderate segmental erosive/necrotizing neutrophilic enteritis, all of which are highly suggestive of a bacterial fetal infection. *Campylobacter* sp. DNA was detected by PCR in the fetal abomasal content and liver. A 734 bp fragment of the 16S rRNA gene amplified from the abomasal content was sequenced for species identification, revealing 99-100% identity with *C. jejuni*, *Campylobacter coli*, *Campylobacter insulaenigrae* or *Campylobacter hepaticus*. Although the *Campylobacter* infecting this fetus could not be unequivocally identified at the species level, DNA sequence analysis ruled out *C. fetus* (Aráoz et al., 2018), and of the four tentative species, *C. jejuni* seems the most likely considering its broadly recognized ability to infect sheep and its proven abortifacient capacity in this species.

Campylobacter spp. are transmitted through the ingestion of food contaminated with feces, vaginal discharges or fetal tissues (Menzies, 2011). Abortion usually occurs at the end of gestation and can affect up to 25% of the flock (Menzies, 2011; Moeller, 2001). Cotyledonary placentitis, multifocal necrotizing hepatitis and suppurative bronchopneumonia

are frequently observed (Moeller, 2001). The diagnosis can be confirmed by bacterial culture, although because of its fastidious growth, other detection methods such as PCR or direct fluorescent antibody test (DFAT) are usually used in diagnostic settings.

Based on our classification criteria, *Cff* is a confirmed cause of abortion in sheep but not goats in Argentina and Uruguay, and a probable cause in Brazil, while *C. jejuni* should be considered a probable cause of abortion in Brazil and Uruguay.

3.1.4. Chlamydia spp.

Chlamydia abortus (formerly *Chlamydophila abortus* and *Chlamydia psittaci* serotype 1) is a zoonotic agent transmitted orally or aerogenously (Rodolakis et al., 1998). Although *C. abortus* causes late gestation abortion, early pregnancy losses and birth of weak lambs have also been described (Rodolakis and Laroucau, 2015).

Antibodies against *C. abortus* have been detected by serologic surveys in sheep and goat flocks with a history of abortions in Argentina (Fiorentino et al., 2015), Brazil (Farias et al., 2013; Rossi et al., 2012; Santos et al., 2012), Chile (Moreira et al., 2017), Colombia (Perry et al., 1980a) and Perú (Sánchez et al., 2018). In one of these studies, a history of abortions in the flock was identified as a risk factor for seropositivity in goats (Santos et al., 2012). These authors tested 975 serum samples from 110 goat herds in Paraíba state, Brazil, using CFT, and found a seroprevalence of 9.3% and 50% at the individual and herd levels, respectively. The frequency of goats with antibodies against *C. abortus* was significantly higher in herds with a history of abortion. No association between the serologic results and history of infertility or stillbirths was found (Santos et al., 2012). Of the other six studies, four did not find an association between reproductive losses and seropositivity (Farias et al., 2013; Fiorentino et al., 2015; Rossi et al., 2012; Sánchez et al., 2018), and the remainder two did not evaluate if such association existed (Moreira et al., 2017; Perry et al., 1980a). In a flock

of goats with abortions in La Rioja (Argentina), a seroprevalence of 19.5% was found by ELISA. *Chlamydia abortus* was not identified by real-time PCR or DFAT in the seven examined aborted fetuses and one placenta, and no microscopic lesions were identified in either the placenta or fetuses (Fiorentino et al., 2015). Therefore, the implication of *C. abortus* in these abortions remained unclear.

In Chile, Moreira et al. (2017) studied the kinetics of anti-*Chlamydia* antibodies by CFT in aborted and non-aborted does for 3 years. The authors concluded that shortly after the abortions occurred, antibody titers reached 1:256 or 1:1,024, but then decreased during the next year and were undetectable by the third year. No information was provided on whether there were differences in seroprevalence among the aborted and the non-aborted cohorts.

Reports of chlamydial abortion are limited to goats from Chile and Argentina (Bedotti et al., 2008; Di Paolo et al., 2019; Saldías et al., 2014). In 2012 the Metropolitan and Biobío regions of Chile had two outbreaks of abortion associated with *C. abortus* in different caprine flocks (Saldías et al., 2014). The first flock presented a high seroprevalence (43.01%, 117/272) as determined by indirect ELISA and CFT, with antibody titers > 1:32. Samples of placenta, lung and liver from two aborted fetuses, one from each flock, were subjected to histopathologic examination and molecular testing. Inflammatory infiltrates were described in the placental stroma and wall of blood vessels, without mention of the presence of intracytoplasmic bacteria (inclusions), which are typical of chlamydiosis although they may be hard to find. *Chlamydia abortus* was amplified by real-time PCR in both cases, while *C. pecorum* and *C. psittaci* were further ruled out by real-time PCR in one set of tissues. Results of testing for other abortifacients were not provided (Saldías et al., 2014).

In La Pampa, Argentina, a presumptive outbreak of chlamydial abortion was described in a goat farm, where late gestation abortions and stillbirths were identified in 30 of 250 Creole goats (Bedotti et al., 2008). Histology revealed necrosuppurative placentitis with

necrotizing vasculitis and isolated foci of nonsuppurative encephalitis in an undetermined number of cases. Six of six aborted and five of nine non-aborted does were seropositive for *Chlamydia* spp. antibodies by ELISA, and all 15 were seronegative for *Brucella* spp. and *T. gondii*. Using this data, we conducted statistical analyses (Fisher´s exact test) and the differences in the proportions of aborted and non-aborted does seropositive for *Chlamydia* spp. were not statistically significant. Fetal tissues or placenta were not tested by PCR, IHC or special stains to detect *Chlamydia* spp. or any other abortifacient that may cause similar or overlapping lesions. Despite describing placental lesions compatible with chlamydiosis, the authors did not comment on whether intracellular bacteria (inclusions) were identified.

In Mendoza, Argentina, Di Paolo et al. (2019) reported reproductive losses in 70/400 Creole goats that either aborted full-term fetuses or delivered weak kids. One stillborn fetus and its placenta were subjected to gross and histopathologic examination. Grossly, the placenta was thickened with a necrotic and fibrinopurulent exudate that was particularly evident in the intercotyledonary regions, while no macroscopic lesions were observed in the fetus. Histologically, the placenta exhibited diffuse necrosis of the villus and intercotyledonary epithelium, infiltration by neutrophils, fewer lymphocytes, plasma cells and macrophages, and mineralization. Trophoblasts were expanded by myriad ~1 μm basophilic inclusions. The chorioallantoic vessels were expanded by a pleocellular inflammatory infiltrate and fibrin (vasculitis) and occluded by fibrin thrombi. The fetus had lymphohistiocytic and neutrophilic perivascular encephalitis and vasculitis, multifocal necrotizing hepatitis with infiltration of macrophages, lymphocytes, and plasma cells along with lymphoplasmacytic cholangiohepatitis, and lymphoplasmacytic interstitial pneumonia. *Chlamydia abortus* organisms were identified in the placenta by a combination of Ziehl-Neelsen stain, and real-time and nested PCRs, and abundant intralesional and

intratrophoblastic chlamydial antigen was demonstrated by IHC. *Toxoplasma gondii* and *C. burnetii* were ruled out by IHC, and *C. pecorum* was ruled out by nested PCR.

Chlamydia pecorum is a normal inhabitant of the digestive tract of ruminants, capable of causing sporadic abortions in sheep and goats (Giannitti et al., 2016; Walker et al., 2015). In 2010, samples of placenta, lung and liver from an aborted caprine fetus from Atacama, Chile were submitted to the OIE reference laboratory for chlamydiosis (Friedrich-Loeffler-Institut, Jena, Germany), where *C. pecorum* was identified by real-time PCR and microarrays (Saldías et al., 2014). No information was provided about the pathologic examination of these tissues or further testing to assess for other possible abortifacients.

Salient lesions of chlamydiosis include neutrophilic and necrotizing placentitis with intratrophoblastic inclusions, representing chlamydial elementary and reticulate bodies, and vasculitis. A multifocal necrotizing hepatitis with mononuclear portal infiltrates can also be identified in some animals (Moeller, 2001). Furthermore, fibrinosuppurative enteritis with intracytoplasmic bacteria in enterocytes has been reported in goat fetuses aborted by *C. pecorum* (Giannitti et al., 2016). *Chlamydia* spp. can be demonstrated by special stains (Giménez, Ziehl-Neelsen or Giemsa) in placental smears or histologic sections. Furthermore, DFAT and IHC can aid in detecting *Chlamydia* spp. antigens (Rodolakis and Laroucau, 2015). Since chlamydial isolation requires cell cultures, molecular detection is recommended (Rodolakis and Laroucau, 2015). This allows easier species identification which is not always possible through immunological assays due to cross-reactivity (Giannitti et al., 2016).

According to our classification criteria, *C. abortus* is a confirmed abortifacient in goats in Argentina, a probable cause of abortion in goats in Chile and Brazil, and a possible cause of abortion in sheep in Argentina, Brazil, Chile, Colombia and Perú. *Chlamydia pecorum* is a probable cause of caprine abortion in Chile; this bacterium has not been identified in sheep or goats in other South American countries.

3.1.5. Coxiella burnetii

Coxiella burnetii is a zoonotic obligate intracellular bacterium with an extremely low infective dose. Late gestation abortion, premature births and stillbirths are usually the only clinical signs of the infection in ruminants (Agerholm, 2013).

Despite evidence of coxiellosis in several species (including humans) from South America (de Oliveira et al., 2018; Oropeza et al., 2010; Perry et al., 1980b; Trezeguet et al., 2010; Troncoso et al., 2014), reports confirming abortions in sheep or goats are lacking in this subcontinent. Abortion and detection of *C. burnetii* by molecular and serological methods was reported in goats in Alagoas, Brazil (de Oliveira et al., 2018). A flock of 332 does, 40 (12%) of which had aborted, was evaluated. Antibodies were identified in 55.1% of 312 tested does using a commercial ELISA kit. Of 27 aborted does tested, 15 (55.5%) were seropositive, this percentage was similar to the overall seroprevalence of the flock. *Coxiella burnetii* DNA was detected by nested PCR following by sequencing in two of 23 tested samples of placenta belonging to four aborted and 19 non-aborted does. Both PCR-positive samples were from aborted and seropositive does. Unfortunately, none of the 23 placentas was subjected to histopathologic examination and other abortifacient agents were not investigated. In Cesar, Colombia, 66 milk samples from ewes and 328 vaginal swabs from goats from 15 herds were analyzed by PCR for the identification of *C. burnetii* DNA. Six percent (4/66) of the milk samples and 0.6% (2/328) of the vaginal swabs were positive (Contreras et al., 2018). In this study, no association with abortions was sought.

Of all South American countries, *C. burnetii* has only been confirmed as an abortifacient in dairy cattle in Uruguay, in one farm in the department of Colonia (Macías-Rioseco et al., 2019) and one farm in the department of San José (Macías-Rioseco et al., 2020). One of these farms is located ~3.5 km away from a large sheep operation and ~10 km

away from a small caprine dairy flock (Giannitti F, personal observation). In rural areas, airborne dispersal of *C. burnetii* from livestock is likely. The bacterium can be transported up to 18 km depending on wind speed, with highest infection risks occurring within 5 km from sources (Clark and Soares Magalhães, 2018). Thus, cases of abortion in other ruminant species are likely in this and perhaps other countries of South America and are probably underreported.

There is serologic evidence of exposure to *C. burnetii* in sheep and goats from Argentina (Romaña, 1962; Trezeguet et al., 2010), Brazil (de Souza et al., 2018), Chile (Troncoso et al., 2014), Colombia (Perry et al., 1980b), Venezuela (Oropeza et al., 2010) and Uruguay (Bacigalupi et al., 1958). In Uruguay, a serologic survey in 591 sheep submitted to a slaughterhouse from 10 of the 19 departments of the country, revealed a seroprevalence of 10.3% by agglutination test. Seropositive individuals were detected from the 10 departments, indicating a widespread geographic distribution of the agent (Bacigalupi et al., 1958).

In the diagnostic investigation of abortive coxiellosis, the placenta is the main and most often only diagnostically useful tissue, exhibiting severe inflammation, necrosis and abundant intratrophoblastic basophilic bacteria (Agerholm, 2013; Moeller, 2001). Molecular detection or IHC in the placenta in the presence of compatible lesions confirm the etiologic diagnosis (Agerholm, 2013; Moeller, 2001). In cases of abortion, serology is not very useful as seropositive but asymptomatic animals can be found and conversely, acutely infected animals can abort before antibodies raise to detectable levels (OIE, 2018).

Based on our classification scheme, *C. burnetii* is a probable cause of abortion in goats in Brazil, and a possible cause of abortion in sheep and goats in Argentina, Brazil, Chile, Colombia, Venezuela and Uruguay, where serologic exposure has been reported.

3.1.6. Leptospira spp.

Even though leptospirosis affects a wide spectrum of animal species and humans, small ruminants have a low susceptibility and are not considered important reservoirs (Leon-Vizcaino et al., 1987). Despite this, there is serologic evidence of exposure to several *Leptospira* serogroups/serovars in sheep and goats in Argentina (Brihuega et al., 1984; Martin et al., 2014; Martinez et al., 2013; Robles et al., 2014), Brazil (Cortizo et al., 2015; Higino et al., 2013; Martins et al., 2012a), Bolivia (Ciceroni et al., 1997), Guyana (Motie and Myers, 1986), Perú (Bautista et al., 2014; Flores et al., 2009), Ecuador (Navarrete, 2019), Colombia (Parra Solano et al., 2016), Venezuela (Valeris-Chacín et al., 2012) and Chile (Zamora et al., 1999). Little information is available on the serovars that impair reproductive performance in sheep and goats worldwide, although serogroups Hebdomadis, Australis and Pomona were identified as possibly involved in abortion, stillbirth and neonatal death in sheep in Northern Ireland (Ellis et al., 1983).

In a caprine flock of unknown size from Guyana that exhibited abortions, stillbirths and death of adult does, four (3.6%) of 112 sampled does showed anti-leptospiral antibodies by microscopic agglutination test (MAT); the seroprevalence in surviving does raised to 46% (17/31) a year later, with titers ranging from 1:200 to 1:1,600 against multiple serovars (Icterohaemorrhagiae, Pyrogenes, Pomona, Hardjo and Wolffi) (Motie and Myers, 1986). Although this indicates exposure to multiple *Leptospira* serovars with rising seroprevalence overtime, the causative role of this agent in the abortions and stillbirths was undetermined.

In Junín, Perú, a case-control study attempted to associate abortions with serologic status in ewes (Flores et al., 2009), but the seroprevalence assessed by MAT did not differ significantly between cases (63/220; 28.6%) and controls (46/220; 20.9%). Serovars Ballum and Icterohaemorrhagiae were frequently detected. In Espírito Santo, Brazil, Cortizo et al. (2015) surveyed 12 ovine and 12 caprine farms and found seroprevalences of 10.4% (46/442) and 11.1% (33/296) in sheep and goats, respectively; Icterohaemorrhagiae was the most

frequent serovar in both livestock species. A statistically significant association was found between seropositivity and abortion, as seroreactive ewes (relative risk: 1.3) and does (relative risk: 1.9) were more likely to belong to herds with a history of abortion than seronegative animals ($p < 0.05$). To our knowledge, this represents the only indirect evidence of a probable abortifacient role of *Leptospira* spp. in South American sheep and goats and suggests that leptospirosis may be an underdiagnosed cause of abortion in domestic small ruminants in the region.

Leptospirosis was investigated as a cause of reproductive losses in a herd of 125 Saanen goats free of brucellosis in Rio de Janeiro, Brazil. Out of 50 pregnant does, 22 late-term abortions, six embryonic resorptions determined by ultrasonography, and two neonatal deaths were reported between August and October of 2009 (Martins et al., 2012b). An unspecified number of necropsied fetuses exhibited jaundice and petechiae in the liver and kidneys, while 15 aborted fetuses (62.8%) were macerated (Martins et al., 2012b). Seropositivity (MAT titers ≥ 1:200) to *Leptospira* spp. was evidenced in 48.8% (61/125) of the goats, mainly to serovar Icterohaemorrhagiae (65.8%), followed by Hardjo and Bratislava (17.1% each), with titers ranging from 1:200 to ≥ 1:800. Urine was collected from all 50 does (48 of which were seropositive) and processed for leptospiral culture and PCR. Although no isolates were obtained, 48/50 (96%) does tested PCR-positive, including 47 seropositive and one seronegative animals. All 22 aborted does tested PCR-positive in urine. The authors inferred that leptospirosis was the cause of the abortions. However, we question that the investigation does not necessarily provide confirmatory evidence of causality as: 1- the % of seropositivity and antibody titers were not compared between aborted (cases) and non-aborted (control) does; 2- samples of the aborted fetuses/placentas were not processed for detection of *Leptospira* spp. DNA (PCR, qPCR), antigens (DFAT, IHC), bacteria (culture, dark-field microscopy, special stains) or anti-leptospiral antibodies (MAT) to investigate fetal

infection or exposure; 3- fetal tissues were not examined histologically to assess for microscopic lesions compatible with leptospirosis; and 4- other common abortifacient agents of goats were not investigated in the does or fetuses.

Leptospira spp. was isolated from a dairy doe in Rio de Janeiro, Brazil (Lilenbaum et al., 2007) that belonged to a flock with reproductive disorders different from abortion (estrous repetition and low conception rates); the isolate was obtained from a 4-year-old goat with no clinical signs, and presumptively classified as serogroup Grippotyphosa by MAT. Isolation or molecular detection of *Leptospira* spp. has never been reported from aborted goats or sheep or their fetuses or placentas in South America, thus direct evidence of fetoplacental infection is lacking in the reviewed literature.

Fetuses aborted by *Leptospira* spp. may exhibit jaundice, subcutaneous petechiae, blood-tinged fluids in the body cavities, generalized visceral congestion and hepatomegaly (Leon-Vizcaino et al., 1987). Microscopically, tubulointerstitial nephritis is the main pathological finding, canalicular cholestasis, and chorioallantoic stromal edema and lymphocytic infiltration may be observed (Moeller, 2012). The spirochetes can be observed in the proximal renal tubules in histologic sections stained by the Warthin-Starry stain, and leptospiral antigen can be demonstrated by IHC in tissue sections, and/or by DFAT in imprints of fetal tissues (kidney, liver, lung, placenta) or smears of fetal fluids (abomasal content, aqueous humor) (Ellis, 2015; Moeller, 2012; Schlafer and Foster, 2016). Leptospiral DNA can be detected using PCR or qPCR with higher sensitivity; culture is not routinely performed in fetuses as it is time-consuming and challenging (OIE, 2018).

Based on our classification criteria, *Leptospira* spp. should be considered a probable cause of abortion in sheep and goats in Brazil, and a possible cause of abortion in Argentina, Bolivia, Guyana, Perú, Ecuador, Colombia, Venezuela and Chile.

3.1.7. Listeria spp.

Listeriosis can affect many species, although most clinical cases occur in ruminants (OIE, 2018). *Listeria monocytogenes* and *Listeria ivanovii* are transmitted by the oral route, cross the placenta after bacteremia, and cause abortion usually in late pregnancy (Fentahun and Fresebehat, 2012; Moeller, 2001).

Recently, an abortion by *L. ivanovii* was reported in a sheep flock in Santa Fe, Argentina (Della Rosa et al., 2019). Ten of 390 Santa Inés ewes aborted full-term fetuses over a period of one month. A fetus aborted at 130 days of gestation exhibited multifocal necrotizing hepatitis, suppurative bronchopneumonia, diffuse meningitis and occasional foci of gliosis in the brainstem and spinal cord. Intralesional bacterial colonies were described in the liver, lungs and meninges. *Listeria ivanovii* was isolated on pure culture from the placenta, brain, liver, lung and abomasal content. Multiple bacterial colonies were immunolabelled in sections of lung, liver and meninges by IHC using a commercial polyclonal antiserum against *L. monocytogenes* serotypes 1 and 4, which calls into question the specificity of the antiserum. Fetal tissues were negative for *T. gondii* and *Neospora caninum* DNA by PCR, and fetal fluids were negative for antibodies against these protozoa by indirect fluorescent antibody test (IFAT).

The etiologic diagnosis of listeriosis is usually based on bacterial identification either through isolation in pure culture (Fentahun and Fresebehat, 2012) or PCR in fetal tissues or placenta with typical lesions. The most consistent lesions in abortions include necrosuppurative cotyledonary and intercotyledonary placentitis, multifocal random necrosuppurative hepatitis and suppurative bronchopneumonia (Moeller, 2001). Visualization of intralesional gram-positive bacilli in tissue sections also aids in the diagnosis (Moeller, 2012). In addition, the bacteria can be detected by IHC (Moeller, 2012).

According to our classification criteria, *L. ivanovii* is a confirmed cause of abortion in sheep in Argentina. *Listeria monocytogenes* has been identified causing neurologic disease in sheep and goats in Argentina (Olguin Perglione et al., 2018) and Brazil (Rissi et al., 2010, 2006); therefore, it should be considered a possible cause of abortion in small ruminants in these countries.

3.1.8. Histophilus ovis, *Actinobacillus seminis* and other bacteria

These bacteria have been classically associated with epididymitis in rams and sporadic abortions in ewes (Moeller, 2012). In South America, *Actinobacillus seminis*, *Histophilus ovis*, *Corynebacterium pseudotuberculosis*, *Staphylococcus aureus*, *Trueperella pyogenes* and *Yersinia pseudotuberculosis* have been isolated from semen of rams with epididymitis (Robles and Olaechea, 2001; Zamora et al., 1977). However, published reports of abortions caused by these agents in small ruminants are lacking in the region.

Many other bacteria can cause sporadic abortions in small ruminants (Kirkbride, 1993; Moeller, 2012, 2001). Generally, abortions occur during mid and late gestation (Moeller, 2012). In Buenos Aires, Argentina, *Bacillus licheniformis* was associated with an abortion in a Saanen dairy goat (Fiorentino et al., 2014). A 60-day-old fetus exhibited non-suppurative abomasitis, mixed enteritis, multifocal hepatitis and non-suppurative pericarditis. *Bacillus licheniformis* was isolated in pure culture from samples of liver, lung and abomasal content. *Toxoplasma gondii* was ruled out by PCR on fetal tissues and IFAT on fetal serum.

Staphylococcus spp. was isolated from an aborted goat fetus in Uruguay (Preliasco et al., 2013). No evidence of gross or histological lesions in the fetus or placenta were provided, therefore, the causal implication of *Staphylococcus* spp. in the abortion was uncertain. Although Uruguay has officially notified the presence of *Salmonella abortusovis* according to an OIE performance of veterinary services evaluation report (Hutter et al., 2014), we did not

find published reports of abortions caused by this agent in any South American country in the reviewed literature.

Pathological findings vary according to the acting bacteria, although necrosuppurative placentitis and suppurative bronchopneumonia are common nonspecific lesions (Kirkbride, 1993; Moeller, 2012). The following guidelines can aid in the diagnosis of sporadic abortion by miscellaneous bacteria (Borel et al., 2014; Kirkbride, 1993; Moeller, 2012):

1- The bacteria must be isolated in large quantities and/or in pure culture in fetal abomasal content, tissues and/or placenta.

2- Pathologic examination should reveal histologic lesions in the placenta or fetal tissues compatible with a bacterial etiology, more often suppurative inflammation or pleocellular inflammation with numerous neutrophils and macrophages.

3- Other specific causes of abortion should be ruled out.

3.2. Protozoa

3.2.1. Toxoplasma gondii

Toxoplasmosis is a major cause of abortion in sheep and goats (Moeller, 2012, 2001). Transmission is mainly horizontal via ingestion of fodder or water contaminated with oocysts shed by felids. Transplacental (vertical) transmission is less frequent (Buxton et al., 2007).

Toxoplasmosis has been diagnosed in most countries in South America, however, there are relatively scarce clinical reports in sheep or goats (Caldeira et al., 2011; de Moraes et al., 2011; Freyre et al., 1987, 1994; Gual et al., 2018; Pescador et al., 2007a; Unzaga et al., 2014). In Uruguay, Freyre et al. (1987) isolated *T. gondii* from a flock of 470 ewes and 30 yearlings, where 10 aborted fetuses and 50 deceased lambs were identified. One placenta and seven aborted fetuses were presented for diagnostic examination. Multifocal non-suppurative and necrotizing encephalomyelitis with gliosis and occasional protozoan cysts were seen in

all the examined fetuses. Likewise, the placenta exhibited histiocytic and plasmacytic inflammation with multiple foci of necrosis and mineralization. *Toxoplasma gondii* was isolated from the placenta. Specific anti-*T. gondii* antibodies were detected by indirect hemagglutination (IH); the seroprevalence was significantly higher in aborted (~35%) than non-aborted (17%) ewes, and aborted ewes had higher antibody titers (up to 1:2,048) than non-aborted ones (1:256).

In Argentina, Unzaga et al. (2014) isolated *T. gondii* from the brain of an aborted caprine fetus, which was seronegative by IFAT. Histologically, the placenta showed necrosis and mineralization, and *N. caninum* infection was ruled out. The *T. gondii* isolate was identified as atypical or non-canonical by nested-PCR-restriction fragment length polymorphism. Gual et al. (2018) reported an outbreak of toxoplasmosis in a flock of Texel sheep on a farm in Buenos Aires province, Argentina. In a group of 184 pregnant sheep, 15 abortions and nine stillbirths were identified. Non-suppurative myocarditis and epicarditis, portal hepatitis, and multifocal necrotizing encephalitis with protozoal cysts were diagnosed histologically in two mummified fetuses. *Toxoplasma gondii* was detected by PCR in both fetuses and by IHC in one. Other causes of abortion including *N. caninum* were ruled out, and a statistically significant association between seropositivity and abortion/stillbirth was established.

In Pernambuco, Brazil, de Moraes et al. (2011) detected *T. gondii* DNA by nested PCR in ovine placentas and fetal tissues. Three abortions and two stillborn lambs out of 35 tested positive by nested PCR. Necrotizing and mononuclear placentitis with areas of mineralization, compatible with toxoplasmosis, were found in the five PCR-positive cases. Although *T. gondii* is the most likely etiology of abortion in these cases, fetal histology was not performed and other causes of abortion, particularly *N. caninum* that can cause similar placental lesions, were not ruled out.

Pescador et al. (2007a) diagnosed one abortion, four stillbirths and one neonatal death in a goat flock from Rio Grande do Sul, Brazil, based on the observation of microscopic lesions and *T. gondii* DNA detection by nested PCR. Immunohistochemistry for *T. gondii* was positive only in one of the six cases, although all the examined specimens had typical lesions of toxoplasmosis, such as multifocal necrotizing encephalitis, mononuclear myocarditis, non-suppurative interstitial pneumonia and periportal mononuclear hepatitis. Does were screened serologically for *T. gondii* using IH; antibody titers up to 1:2,048 were found. Other causes of abortion including *N. caninum* and bacteria were ruled out.

Caldeira et al. (2011) reported abortions in a Saanen flock from Mato Grosso, Brazil. Seven fetuses aged 110 to 140 days gestation were submitted for diagnostic investigation. All of them showed encephalitis, and one exhibited mononuclear pneumonia. *Toxoplasma gondii* DNA was detected in the seven fetuses using nested PCR. In addition, four aborted does presented high antibody titers against *T. gondii* by IFAT, ranging from 1:1,024 to 1:32,768. Other abortifacients were ruled out through bacterial culture, and PCR for *B. abortus* and *N. caninum*.

Serologic and statistically significant evidence of abortion has been reported in sheep and goats in Brazil (Brandão et al., 2009; Cosendey-KezenLeite et al., 2014; Maia et al., 2021; Pimentel et al., 2013; Romanelli et al., 2007) and Argentina (Gos et al., 2016; Gual et al., 2018). In Paraná, Brazil, 305 ewes from 9 farms were screened by IFAT (Romanelli et al., 2007). Ewes reported with reproductive problems during the first and second third of pregnancy were more likely to be seropositive for *T. gondii* than ewes in the last third of gestation. Gos et al. (2016) found a statistically significant association between abortions and seroreactivity in goats from La Rioja, Argentina, where approximately 22.4% (53/237) of does were seropositive by IFAT. Aborted does presented antibody titers up to 1:800, and 68% of the analyzed flocks had seropositive animals. Other serologic surveys performed in sheep

and goats in Brazil (Nunes et al., 2013; Rizzo et al., 2018), and sheep in Perú (Reif et al., 1989) did not find associations between reproductive failure and serologic results. Furthermore, anti-*T. gondii* serum antibodies have been detected in sheep in Chile (Gorman et al., 1999), sheep and goats in Colombia (Alvarez et al., 2017; Martínez-Rodriguez et al., 2020; Perry et al., 1979) and goats in Venezuela (Nieto and Meléndez, 1998).

The finding of multifocal necrotizing and non-suppurative encephalitis in aborted fetuses is highly suggestive of a protozoal abortion (Dubey, 2010). Immunohistochemical techniques or PCR can be used to accurately identify *T. gondii* (OIE, 2018). Additionally, antibodies can be detected by IFAT or ELISA in maternal serum and/or fetal fluids (Dubey, 2010), even though they can prove exposure, they do not necessarily prove causality.

Based on our classification criteria, *T. gondii* is a confirmed cause of abortion in sheep and goats in Argentina, Brazil and Uruguay, and a possible cause of abortion in Chile, Colombia, Perú and Venezuela.

3.2.2. Neospora caninum

Neospora caninum affects mainly cattle, while sheep and goats are less commonly affected (Dubey, 2003). Unlike toxoplasmosis, the main route of transmission of *N. caninum* is transplacental (González-Warleta et al., 2018). Abortion in mid or late pregnancy is usually the only clinical sign in adult ewes and does (Moeller, 2012). In these species, *N. caninum* usually causes sporadic abortions/stillbirths, although more significant losses affecting groups of sheep were described in a flock in Spain (González-Warleta et al., 2014).

Abortions by *N. caninum* have been confirmed in goats and sheep from Argentina (Campero et al., 2018; Hecker et al., 2019) and Brazil (Costa et al., 2014; Pinto et al., 2012). Pinto et al. (2012) identified *N. caninum* in two ovine fetuses of a flock of 268 Santa Inés sheep that had contact with dogs and dairy cows in Mato Grosso do Sul, Brazil. Both fetuses

showed microscopic lesions of non-suppurative myocarditis and multiple foci of mononuclear cell infiltration and gliosis with protozoal structures in the brain. *Neospora caninum* antigen was identified by IHC in the myocardium and brain. The authors also described a weak positive labeling for *T. gondii* and interpreted that this was probably a cross-reaction given that polyclonal antibodies were used as a primary antibody. Unfortunately, molecular, ultrastructural, or serological tests to further identify the infecting protozoa were not performed.

Costa et al. (2014) assessed caprine fetuses from Minas Gerais, Brazil, naturally infected with *N. caninum* to characterize the inflammatory reaction in the brain. Of eight analyzed fetuses aborted at 90-150 days of gestation, two had multifocal necrotizing encephalitis with gliosis and inflammation, and four exhibited multifocal gliosis and/or mononuclear perivascular cuffs, while no lesions were detected in the remainder two fetuses. Protozoal cysts were observed in the thalamus or cerebral cortex of four fetuses. Etiologic confirmation was achieved by IHC and PCR, while *T. gondii* was ruled out.

Caprine abortion caused by *N. caninum* was diagnosed in a dairy herd from Buenos Aires province, Argentina (Campero et al., 2018). A fetus aborted at 3 months of gestation exhibited necrotizing myocarditis and hepatitis, interstitial pneumonia and nephritis. *Neospora caninum* was detected by IHC in the myocardium and by PCR in the heart, liver, lung, kidney and skeletal muscle. Maternal serology revealed an anti-*N. caninum* antibody titer of 1:3,200 by IFAT; however, no antibodies to the protozoa were detected in the fetus. Fetal tissues were negative for *T. gondii* DNA by PCR.

Recently, Hecker et al. (2019) diagnosed neosporosis in an aborted ovine fetus of 112 days of gestation in Buenos Aires province, Argentina. In this flock, abortions were identified in 10/119 (8.4%) ewes. The diagnosis was based on pathological findings, which included necrotizing placentitis, lymphohistiocytic myo-, endo- and epicarditis and glossitis, coupled

with immunohistochemical identification of *N. caninum* antigen in the placenta and tongue, *N. caninum* DNA detection from brain, lung and heart, and IFAT titers (1:800) in fetal fluids. Other causes of abortion, including *T. gondii*, were ruled out by IHC and bacterial cultures.

A few surveys associated *N. caninum* serology with abortions in sheep in Argentina and Brazil (Hecker et al., 2019; Machado et al., 2011; Salaberry et al., 2010). Anti-*N. caninum* antibodies were detected in ovine flocks in Colombia (Patarroyo et al., 2013) and Uruguay (Suzuki et al., 2011).

Fetuses aborted by *N. caninum* show microscopic lesions morphologically indistinguishable from those caused by *T. gondii* (Barr et al., 1990). For this reason, methods such as IHC or PCR on fetal tissues and placenta must be used to identify the etiological agent (Dubey and Schares, 2006). Serological examination of aborted and healthy ewes/does and fetuses may aid in the diagnosis (Dubey and Schares, 2006).

Based on our classification criteria, *N. caninum* is a confirmed cause of abortion in sheep and goats in Argentina and Brazil, and a possible cause of abortion in sheep in Colombia and Uruguay, where serologic exposure was reported.

3.2.3. Sarcocystis spp.

Sarcocystis spp. rarely causes clinical disease in ruminants. However, sporadic abortions and stillbirths have been reported in small ruminants (Dubey et al., 2016). The most pathogenic species for sheep and goats are *S. tenella* and *S. capracanis*, respectively (Moeller, 2012). Both species have been identified by morphological, ultrastructural and molecular methods in ovine and caprine samples obtained from abattoirs in Brazil (Bittencourt et al., 2016). Pescador et al. (2007b) reported a stillborn lamb infected by *Sarcocystis* spp. in a sheep flock in Brazil. In this case, eight of 60 Corriedale ewes had late gestation abortions and stillbirths. The lamb exhibited multifocal necrotizing encephalitis and

non-suppurative myocarditis. Rosette-forming protozoal schizonts were detected in the vascular endothelium of the cerebrum, lungs and kidneys. The external membrane of the schizonts was weakly positive for the periodic acid-Schiff (PAS) reaction, but merozoites and nuclei were PAS-negative. Immunohistochemistry for *T. gondii* was negative, while *N. caninum* IHC was positive and interpreted as a cross-reaction due to the use of polyclonal antibodies. Finally, *Sarcocystis* spp. was identified through transmission electron microscopy, that revealed absence of rhoptries and parasitophorous vacuoles. Molecular testing and *Sarcocystis* spp. IHC were not pursued.

A longitudinal serologic study in a farm located in Buenos Aires, Argentina, found anti-*Sarcocystis* antibodies in 93/129 (72.09%) ewes and 57/117 (67.57%) lambs. *Sarcocystis tenella* was detected in the skeletal muscle of a lamb by PCR and DNA sequencing (Hecker et al., 2018). In Perú, Castro and Leguía (1992) analyzed samples of heart and esophagus from 134 sheep and 63 goats by trichinoscopy. Intrasarcoplasmic protozoal cysts morphologically resembling *Sarcocystis* spp. were found in the heart of 91% and 52.4% sheep and goats, respectively, and the esophagus of 39.8% of the sheep and 76.2% of the goats. This evidence indicates that sarcocystiosis is present in sheep and goats in these countries.

The diagnosis of abortion by *Sarcocystis* spp. is based on the detection of compatible lesions and characteristic protozoal structures in the endothelial cells, coupled with IHC, molecular (Moeller, 2012), and/or ultrastructural identification of the parasites.

Based on our classification criterion, *Sarcocystis* spp. is a confirmed cause of abortion in sheep in Brazil, and a possible cause of abortion in Argentina and Perú.

3.2.4. Trypanosoma vivax

Trypanosoma vivax is a flagellate protozoan that causes acute or chronic systemic disease in ruminants (OIE, 2018). It has been reported in all South American countries, except for Uruguay and Chile (Dávila and Silva, 2006; Jones and Dávila, 2001). Transmission occurs mainly through hematophagous insects, such as *Tabanus* spp. and *Stomoxys* spp. (Dávila and Silva, 2006; Galiza et al., 2011). Despite its wide distribution in South America, there are no reports of confirmed abortions by *T. vivax* in the region, although an indirect association has been established (Batista et al., 2009; Galiza et al., 2011). Batista et al. (2009) surveyed 177 goats and 248 sheep from four farms in Paraíba, Brazil, and found a prevalence close to 25% in both species by direct examination of blood smears (buffy coat) and PCR. Infected goats and sheep showed apathy, pale mucous membranes, weakness, weight loss, abortions, and gave birth to weak, poor performing neonates that died within three days. A second survey performed five months later found that the disease had evolved into a chronic and asymptomatic form. Although abortions occurred during the outbreaks of trypanosomosis, neither fetal tissues nor placentas were analyzed. Thus, definitive confirmation of causality is lacking (Batista et al., 2009).

A severe outbreak of trypanosomosis was reported in sheep in Paraíba, Brazil (Galiza et al., 2011). Of 306 ewes, 240 exhibited clinical signs and 216 died within four months. Anorexia, lethargy, anemia, tremors, head pressing and abortions were observed. Approximately 75% of the infected ewes aborted or gave birth to weak lambs that died soon thereafter. *Trypanosoma vivax* was identified in blood smears and detected by PCR on blood samples obtained from symptomatic and asymptomatic ewes in successive surveys. All symptomatic sheep (32/32) were anemic, 52.17% (12/23) were PCR-positive for *T. vivax* and in 75% (24/32) the parasite was observed on blood smears. Asymptomatic animals were not anemic (0/2), and the parasite was not visualized in blood smears (0/2), although *T. vivax* DNA was detected by PCR in 46.7% (35/75) of them. Necropsy of three affected sheep

revealed enlarged lymph nodes and spleen, serous atrophy of pericardial fat, watery blood and transparent fluid in the abdominal and thoracic cavity. Microscopically, multifocal lymphoplasmacytic myocarditis, mononuclear periportal hepatitis with Mott cells, splenic white pulp hyperplasia and severe non-suppurative meningoencephalitis with lymphoplasmacytic vasculitis and areas of malacia were noted. Histologic examination was performed in one aborted fetus and no lesions were found.

T.M.F. Silva et al. (2013a) described late gestation abortions and the birth of premature and weak lambs in ewes experimentally inoculated with *T. vivax* strains isolated by Galiza et al. (2011). Seven examined fetuses had multifocal lymphocytic hepatitis with hepatocellular necrosis, extensive lymphoplasmacytic pericarditis and multifocal lymphocytic encephalitis. In addition, placentitis with trophoblastic necrosis was observed. *Trypanosoma vivax* was detected by PCR in several fetal tissues (brain, heart, kidneys and testicles), fluids (blood, amniotic fluid) and placenta. This study confirmed transplacental transmission of *T. vivax* leading to fetoplacental pathology under experimental circumstances in pregnant sheep with high parasitemia. Whether this occurs under natural conditions has not been explored.

The diagnosis of trypanosomosis is based on the detection of the parasite by PCR or direct examination techniques (e.g., Giemsa-stained blood smears) (OIE, 2018). ELISA and IFAT techniques are other approved methods for identification of this organism (OIE, 2018).

Based on our classification criteria, and the cited experimental infection, *T. vivax* could be regarded as a possible cause of abortion in sheep and goats in most South American countries, except for Chile and Uruguay. However, it should be stated that cases of abortion with fetoplacental infection by this agent have not been confirmed under natural conditions.

3.3. Viruses

3.3.1. Bluetongue virus

Bluetongue virus (BTV, *Orbivirus*) is widespread in South America, mainly in tropical and subtropical regions favorable for vector survival (Lager, 2004), as transmission occurs through the bite of *Culicoides* midges (Maclachlan et al., 2015). Antibodies against BTV have been detected in serum of sheep and goats in Argentina (Lager, 2004), Brazil (Sbizera, 2018), Chile (Tamayo et al., 1985), Ecuador (Merino Mena, 2011), French Guiana (Lancelot et al., 1989), Guyana (Gibbs et al., 1983), Perú (Jurado et al., 2020; Navarro et al., 2019; Rosadio et al., 1984) and Surinam (Gibbs et al., 1983). Although never reported in sheep or goats, serologic evidence of BTV infection has been provided for cattle in Venezuela (Gonzalez et al., 2000) and Colombia (Homan et al., 1985). Bolivia, Paraguay, and Uruguay are the only South American countries where BTV has not been documented in any species. However, since vector competent *Culicoides* spp. have been reported in these countries (Ronderos et al., 2003; Spinelli and Martinez, 1991; Veggiani Aybar et al., 2015, 2011), and the virus circulates in neighboring regions, it is likely that BTV is present in these countries. Of all the serologic studies performed in sheep or goats in South America, only one conducted in Brazil identified that abortion was significantly more frequent in seropositive ewes by risk factor analysis (Sbizera, 2018).

Bluetongue disease outbreaks occurred in seven sheep farms in Rio Grande do Sul, Brazil. In these outbreaks, abortion was recorded in only one sheep (Guimarães et al., 2017). The aborted fetus, of undetermined gestational age, was retrieved from an affected sheep showing apathy, coughing, dysphagia and drooling. A small amount of BTV RNA was detected in the fetal liver by RT-qPCR (cycle threshold 36), but no gross or microscopic lesions were found in the fetus. This suggests that the abortion may have resulted from the ewe's clinical deterioration rather than fetal pathology despite transplacental infection with a low viral load in the fetus. To the best of our knowledge, this is the only documentation of BTV infection in an aborted sheep fetus in South America. Congenital malformations caused

by BTV as described in other regions of the world have not been reported in small ruminants in South America (Maclachlan and Osburn, 2017).

Aborted fetuses usually manifest hydranencephaly that becomes more severe as gestation progresses when fetal infection takes place before mid-gestation (Maclachlan and Osburn, 2017). Microscopically, necrotizing encephalitis with gliosis can be observed (Maclachlan et al., 2009; Moeller, 2012). Detection of viral RNA by RT-qPCR is recommended since virus isolation is challenging (Maclachlan et al., 2015). Virus detection may no longer be possible at the time of abortion in some fetuses with malformations caused by BTV infection in earlier gestational periods. Serological analysis can be attempted through virus neutralization test (VNT), AGID or ELISA (OIE, 2018).

Based on our classification criteria, BTV is a probable cause of abortion in sheep in Brazil, and a possible cause of abortion in most, if not all, other South American countries.

3.3.2. Border disease virus

Border disease virus (BDV, genus *Pestivirus*) is transmitted mainly through direct contact with persistently or temporarily infected individuals (Moeller, 2012). BDV causes embryonic death, abortions and the birth of lambs with nervous signs and fleece defects (Løken, 1995). There are no clinical cases registered in South America and there is no serologic association of BDV with abortions. Its presence has been evidenced through serologic surveys in sheep in Chile (Tadich et al., 1998) and Perú (Alvarez et al., 2002; Llancares et al., 2012). In São Paulo, Brazil, Gaeta et al. (2016) analyzed 268 serum samples from ewes with a history of abortion, finding only two (0.75%) positive individuals by VNT. Unfortunately, differentiation between antibodies against BDV and bovine viral diarrhea virus (BVDV) was not possible. Thus, the seropositive ewes could have been exposed to

either virus. The causative role of BDV in gestational losses in South American flocks remains unknown.

Regarding the diagnosis, fetuses often show hypomyelination in the brain and spinal cord (Moeller, 2012). RT-qPCR is the most sensitive technique for viral detection (OIE, 2018). Antibody detection can be performed by VNT and ELISA (OIE, 2018). In acute infections, testing of sera from convalescent and recently infected animals is recommended (OIE, 2018).

Based on our classification criteria, BDV is a possible cause of abortion in sheep and goats in Brazil, Chile and Perú.

3.3.3. Caprine herpesvirus 1

Caprine herpesvirus 1 is transmitted via the genital and nasal routes (Tempesta et al., 1999). Clinical features include genital lesions, and digestive and respiratory signs, late gestation abortions and neonatal deaths (Moeller, 2012; Uzal et al., 2004).

Despite serological evidence of exposure in goats in Argentina (Echague et al., 2016; Maidana et al., 2015; Suárez et al., 2016, 2015) and Brazil (Borges, 2015; Gregory et al., 2020; M.L.C.R. Silva et al., 2013b), reports of clinical disease or serological association with reproductive losses are lacking.

The diagnosis is based on the detection of histologic lesions in aborted fetuses, including multifocal necrosis with intranuclear viral inclusion bodies in the liver, lungs and kidneys (Moeller, 2012, 2001). It is often difficult to isolate the virus from fetal tissues; hence, PCR is recommended for viral identification (Moeller, 2012; Uzal et al., 2004). Antibody detection can be achieved using VNT or ELISA with similar sensitivity (Marinaro et al., 2010).

Based on our classification criteria, caprine herpesvirus 1 should be considered a possible cause of abortion in Argentina and Brazil.

3.4. Abortifacients not reported in South America

Many abortifacients of sheep and goats have never been reported in South America. Recommended samples, diagnostic methods and salient fetoplacental lesions for some of these agents are summarized in Supplementary Table.

4. Conclusions

Bacterial, protozoal and viral causes of abortion in sheep and goats have seldom been evaluated in South America. The occurrence, prevalence, impact on animal and public health, production and the economy of abortifacients of small ruminants are largely unknown in South America. Campylobacteriosis, chlamydiosis, listeriosis, toxoplasmosis, neosporosis and sarcocystiosis have been confirmed as small ruminant abortifacients in the region. For other diseases that are known to be present in South America, such as brucellosis caused by *B. ovis* and *B. melitensis*, coxiellosis, leptospirosis and bluetongue disease, there is little, mostly seroepidemiologic evidence or fetal infection supporting probable abortion causality in sheep and/or goats. *Yersinia* spp., caprine herpesvirus 1 and pestiviruses infect small ruminants in the region, although they have not been associated with abortion. However, they should be considered possible causes of abortion given their known abortigenic potential as observed in other regions of the world. Other agents such as *Flexispira rappini*, *Francisella tularensis*, *Anaplasma phagocytophilum,* Rift Valley fever virus, Wesselbron disease virus and bunyaviruses, known to be abortifacients for sheep and goats in other regions of the

world, have not been documented in South America. While some of these agents could in fact be exotic in the region, others may have gone undetected.

Major limitations for identifying causes of abortion in sheep and goats in South American countries include 1- the difficulty of acquiring quality samples for laboratory investigation under extensive field conditions, 2- lack of access to veterinary laboratories with reliable diagnostic procedures for the identification of specific pathogens and pathology, particularly in remote or marginal areas with large populations of small ruminants, and 3- financial and strategic limitations for disease monitoring and surveillance programs. These limitations hamper the eventual detection of emerging/re-emerging communicable diseases in South America.

Conflict of interest

The authors declare that they have no conflicts of interest.

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