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Campylobacter isolation from the feces of sheep with a history of reproductive disorders bred in the state of São Paulo, Brazil

Isolamento de Campylobacter em fezes de ovinos com distúrbios reprodutivos pertencentes a criatórios do Estado de São Paulo, Brasil

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Abstract

Campylobacter species are a significant cause of sheep abortion in most sheep-raising countries. The relationship between the presence of *Campylobacter* spp. in fecal samples and reproductive disorders was investigated in 274 sheep from 28 properties in the state of São Paulo, Brazil. Biological samples from 16 aborted fetuses, one uterus, six placentas, five uterine secretion samples, five vaginal swabs, 17 semen samples, and three preputial swabs were also subjected to bacterial isolation. The bacteria were isolated from fecal samples of 14.9% (5/28) of the properties, affecting 3.65% (10/274) of the sheep, 3.5% (9/255) of females and 5.3% (1/19) of males. *Campylobacter jejuni* was the most prevalent species, present in 66.67% (7) of the positive samples, followed by *Campylobacter coli*, present in 22.22% (2), and one strain was identified as *Campylobacter* spp. The birth of "weak" lambs (p=0.06, OR=6.83 and CI=1.73 to 27.05) and neonatal death (p=0.087, OR=3.5 and CI=0.83 to 14.72) were associated with the fecal isolation of *Campylobacter* spp. Diarrhea was also associated with the bacteria (p=0.003, OR=9.83 and CI=2.19 to 44.18). The dissemination of *Campylobacter* spp. in Brazilian sheep is low and that, at present, the existing strains are not responsible for significant economic losses in sheep production, especially in adult animals.
Key words: Campylobacteriosis, *Campylobacter jejuni*, *Campylobacter coli*, and neonatal death

Resumo

Espécies de Campylobacter são uma importânte causa de abortos em ovinos de muitos paíse. No intuito do isolamento de *Campylobacter* spp. em ovinos, foram colhidas 274 amostras fecais de ovinos do Estado de São Paulo, Brasil que possuiam histórico de distúrbios reprodutivos. O isolamento bacteriano ocorreu em 3,65% (10/274) das amostras, correspondendo 3,5% (9/255) das fêmeas e 5,3% (19/01) dos machos. *Campylobacter jejuni* foi a mais prevalente, responsável por 66,67% (7) das amostras positivas

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e o *Campylobacter coli* responsáveis por 22,22% (2) e uma cepa foi identificada como *Campylobacter* spp. Os parâmetros analisados que apresentaram valor de p <0,1 foram o nascimento de cordeiros "fracos" (p=0,06, OR=6,83 e IC=1,73 a 27,05) e morte neonatal (p=0,087, OR=3,5 e IC=0,83 a 14,72). Foi observado que os animais infectados apresentam maior risco de quadros de diarreia (p=0,003, OR=9,83 e IC=2,19 a 44,18). *Campylobacter jejuni* e *Campylobacter coli* estão presentes nos rebanhos paulistas e observou-se associação de ovinos com isolamento bacteriano fecal com quadro de nascimento de cordeiros "fracos" e consequente morte neonatal. Além de sua associação com quadros de diarreia que predispões à infecção oral de cordeiros a bactéria. A disseminação de *Campylobacter* spp. nos rebanhos ovinos Brasileiros é baixa e que, por enquanto, as cepas existentes não são responsáveis por grandes prejuízos na ovinocultura, principalmente em animais adultos.
Palavras-chave: Campilobacterioses, *Campylobacter jejuni*, *Campylobacter coli* e morte neonatal

Introduction

Campylobacter jejuni, *Campylobacter coli* and *Campylobacter fetus* subspecies *fetus* are present worldwide and are the main species of *Campylobacter* that cause reproductive disorders in sheep (HEDSTROM et al., 1987; DIKER et al., 1988; SAHIN et al., 2008). These bacteria can be found in environments such as soil, water and food, most likely as result of contact with contaminant sources such as animal feces and aborted tissues (BROMAN et al., 2002; PITKÄNEN, 2013). May colonize several animal species with the risk of interspecies transmission. Roug et al. (2013) isolated *C. jejuni* and *C. coli* in sheep, goats, cattle and pigs at agriculture fairs in California, USA. Pao et al. (2014) found that sheep pose a greater risk than goats in *C. jejuni* contamination at co-grazing small ruminant farms.

Healthy sheep can act as reservoirs of these bacteria (AÇIK; ÇETINKAYA, 2006; SPROSTON et al., 2010), intermittently excreting them in the pasture, especially in response to stressful situations such as birth, weaning and feeding changes (STANLEY et al., 1998; JONES et al., 1999), however are rapidly inactivated on grassland in the occurrence of high temperatures (MORIARTY et al., 2011). As similar strains are capable of causing abortions in poultry and cattle, these animals are also considered reservoirs of *Campylobacter* spp. (DIKER et al., 2000). The presence of birds on sheep farms increases the risk of lambs becoming infected in their first days of life (BROMAN et al., 2002; PAO et al., 2014).

Scarcelli et al. (1998) studied the feces of healthy sheep in the state of São Paulo and obtained *Campylobacter jejuni* isolates in 2.9% (2/69) of sheep. In Nigeria was obtained 18% (93/518) of Campylobacter isolates from rectal swab in ovine. The Campylobacter species isolated in this study were *Campylobacter jejuni* (79,6%), *Campylobacter coli* (11,8%), *Campylobacter lari* (6,4%) and *Campylobacter upsaliensis* (2,2%) (SALIHU et al., 2009).

In Brazil, the occurrence of these bacteria and their association with reproductive problems in sheep is low. While *Campylobacter jejuni* and *Campylobacter fetus* subspecies *fetus* have been isolated in cases of abortions in Brazil (VARGAS et al., 2005; GRESSLER et al., 2012), these bacteria represent a major cause of abortions in countries like New Zealand with high rates of fetal isolates (BIRD et al., 1984; QUINLIVAN; JOOP, 1982). Even vaccinated animals may have reproductive problems because the bacterium exhibits high variability within the herd, and the immunization is not effective against all strains capable of causing abortions (COLLINS; LISLE, 1984; DELONG et al., 1996; FENWICK et al., 2000).

Campylobacteriosis in sheep are characterized by abortion in the last trimester of gestation, stillbirth, the birth of premature and weak lambs, and occasionally the death of ewes due to metritis (DENNIS, 1975; HEDSTROM et al., 1987;

SAHIN et al., 2008; GRESSLER et al., 2012) and of lambs due to diarrhea and/or gastroenteritis (STANSFIELD et al., 1986). Many sheep have diarrhea before the first episode of abortion, and diarrhea occurs in approximately 10% of the herd (WALT, 1994). Further, occasionally retention of dead fetus is observed which may lead to death of the pregnant ewes due to septicemia and uterine sepsis. However, no clinical signs can be seen in these ewes at the beginning of infection (SKIRROW, 1994).

Artificially inoculated females aborted in 100% of cases, with clinical signs similar to cases of natural infection, characterized by purulent endometritis and necropurulent placentitis with yellow cotyledons and edematous caruncles with hemorrhagic streaks, covered by mucoid-serosanguinous and fibrinous exudate (HEDSTROM et al., 1987). *C. coli*, in Turkey, was isolated and identified from vaginal discharge samples of two sheep and 10,25% of aborted foetus (BÜYÜK et al., 2011). Zan Bar et al. (2008) experimentally inoculated males with *Campylobacter fetus fetus*, observing decreased motility and sperm vigor along with morphological changes, suggesting that this species may be toxic to sperm cells.

Due to the potential of *Campylobacter* spp. to cause reproductive disorders in sheep, the present study aimed to investigate the relationship between reproductive disorders and fecal isolates of these bacteria in sheep from the state of São Paulo.

Materials and Methods

Between the years 2008 and 2009 a total of 274 fecal samples were collected from sheep with a history of reproductive disorders; 255 were collected from females and 19 from males. The animals were from 28 farms located in 19 municipalities of the state of São Paulo, Brazil being them Atibaia, Campinas, Cunha, Indaiatuba, Itapevi, Itatiba, Itu, Itupeva, Jundiaí, Morungaba, Pedra Bela, Piedade, Piracaia, Pirassununga, Santa Rita do Passa Quatro, Santo Anastácio, São Paulo, Sorocaba, Valinhos and Vargem. In addition to the fecal samples, 16 aborted fetuses, one uterus, six placentas, five samples of uterine secretions, five vaginal swabs, 17 semen samples and three preputial swabs were subjected to bacterial isolation of *Campylobacter* spp. All samples were placed in sterile plastic bottles and refrigerated until the time of diagnosis, which occurred within 24 hours after collection.

The bacterial isolation from fecal samples was performed according to Scarcelli et al. (1998). Bacterial isolation from the semen samples was performed according to the method described by Genovez et al. (1999), and the other samples were processed according to Scarcelli et al. (2004).

The reproductive disorders observed in this study were abortion, endometritis, fetal malformation, birth of "weak" lambs, stillbirth, dystocia, premature delivery, the presence of uterine secretions, uterine prolapse, repeat estrus, infertility and/or placental retention in ewes and infertility, orchitis, epididymitis, degeneration, testicular hyperplasia or hypoplasia and/or lack of libido in rams.

All sheep in the present study underwent clinical and gynecological or andrological examination (GRUNERT et al., 2005), and the observed alterations were recorded in individual clinical records. Among the observations in females were hyperpigmented or hypopigmented vaginal mucosa; the presence of nodules, petechiae, pustules, telangiectasias and vesicles in the vaginal region; and the presence of uterine secretions. Males were diagnosed with orchitis, epididymitis and infertility.

The results were analyzed using a multidimensional descriptive technique that analyzed the proportion and number of individuals with each group of disease variables. The groups with significant relationships ($p<0.1$) were evaluated using logistic regression to measure the risk associated with the various model variables (AGRESTI, 2012; PAULINO; SINGER, 2006).

Results

The percentage of fecal samples positive for *Campylobacter* spp. was 3.65% (10/274); 3.5% (9/255) of the samples from females and 5.3% (1/19) of the samples from males. The isolates were obtained from five different herds (5/28), with four samples obtained from one herd, three from a second and only one sample from the remaining farms.

Two species were identified, with *Campylobacter jejuni* as the most prevalent, present in 66.67% (7/10) of the isolates, followed by *Campylobacter coli,* present in 22.22% (2/10). It was not possible to identify the species of the final sample, and it was instead classified by genus-specific tests as *Campylobacter* spp.

Campylobacter jejuni was isolated from the Santa Inês rams. This ram was housed in an individual stall, where its walls and troughs were contaminated with feces from birds that were raised free on the property. No bacteria were isolated from any of the other biological samples subjected to diagnosis.

No relationship was found between the bacterial isolation and the presence of symptoms at the time of gynecological or andrological examination. Referring to the history of reproductive disorders, a significant correlation was observed between the presence of *Campylobacter* spp. and the birth of "weak" lambs, neonatal death and placental retention (Table 1).

Table 1. Analysis of significance (p value) between ewes positive for fecal isolation of *Campylobacter* spp. and the presence of reproductive disorders.

Variables		N	Isolations [n] (%)]	Odds ratio (CI) (95%)	p Value
Abortion	Yes	136	5 (3.7%)	-	0.87
	No	119	4 (3.4%)		
Endometritis	Yes	6	0 (0.0%)	-	-
	No	249	9 (3.6%)		
Fetal malformation	Yes	2	0 (0.0%)	-	-
	No	253	6 (2.7%)		
Neonatal death	Yes	33	3 (9.1%)	3.5 (0.83-14.72)	0.087
	No	222	6 (2.7%)		
Birth of weak lamb	Yes	29	4 (13.8%)	6.83 (1.73-27.05)	0.006
	No	226	5 (2.2%)		
Stillbirth	Yes	7	0 (0.0%)	-	-
	No	248	9 (3.6%)		
Dystocia	Yes	6	1 (16.7%)	-	0.12
	No	249	8 (3.2%)		
Premature delivery	Yes	3	0 (0.0%)	-	-
	No	252	9 (3.6%)		
Uterine prolapse	Yes	2	0 (0.0%)	-	-
	No	253	9 (3.6%)		
Repeat estrus	Yes	77	0 (0.0%)	-	-
	No	178	9 (5.1%)		
Placental retention	Yes	3	1 (33.3%)	15.88 (1.26-187.61)	0.032
	No	252	8 (3.2%)		

Ref.: Reference parameter for the analysis of significance. Values of $p \leq 0.1$ indicate statistical significance.

Among the signs observed during the clinical examination, a significant relationship was also observed between animals with diarrhea and isolates of *Campylobacter* spp. (p=0.003), with a risk factor of 9.83 (2.19-44.18).

Discussion

Among the three species of *Campylobacter* capable of causing reproductive disorders in sheep, *Campylobacter jejuni, Campylobacter coli* and *Campylobacter fetus fetus* (HEDSTROM et al., 1987; DIKER et al., 1988; SAHIN et al., 2008; BÜYÜK et al., 2011), only the latter was not isolated in this study of the herds of São Paulo. However, the same species were also isolated in previous studies in healthy animals (STANLEY et al., 1998; JONES et al., 1999). Both species were isolated in sheep, goats and cattle healthy in USA (ROUG et al., 2013). Should be borne attention because the *C. coli* and *C. jejuni* isolates in this study have a zoonotic character, and are associated with acute gastrointestinal disease and septicemia in humans (STEPHEN, 2013).

The percentages of isolates (3.65%) observed in the present study were similar to those obtained in Brazil by Scarcelli et al. (1998), but lower than those in the studies conducted mainly in New Zealand, United States and Nigeria, where the bacteria has a greater epidemiological impact (QUINLIVAN; JOOP, 1982; BIRD et al., 1984; MANNERING et al., 2004, 2006; SALIHU et al., 2009). Between 2006 and 2009, in New Zealand, sera were tested for *C. fetus fetus*, 1,644/3,429 (48%) were positive, only 34/298 (11%) flocks tested for were completely seronegative (DEMPSTER et al., 2011)

Birds were observed at 7/28 properties, and although the presence of birds is an aggravating factor for infection by *Campylobacter* spp. (BROMAN et al., 2002; PAO et al., 2014), a significant relationship with the disease was not observed in this study. However, the *Campylobacter jejuni* infection of the Santa Inês ram likely occurred orally because its stall was heavily contaminated with the feces of birds that roamed free. The ram had a history of low fertility after the breeding season, which may have been influenced by the bacterial toxicity of sperm cells (ZAN BAR et al., 2008), but the bacteria could not be isolated from the semen samples from this ram.

Regarding the history of reproductive disorders, the results corroborate those of other authors associating the infection with the birth of "weak" lambs and subsequent neonatal death (DENNIS, 1975). However, no association was observed between infection and abortion, presenting a different picture from that reported in countries like New Zealand (QUINLIVAN; JOOP, 1982; BIRD et al., 1984; MANNERING et al., 2006), where prophylactic measures such as vaccination have been observed to decrease cases of miscarriage (BIRD et al., 1984; FENWICK et al., 2000; MANNERING et al., 2004). This measure has not yet been adopted in Brazilian herds because abortions occur sporadically in sheep (VARGAS et al., 2005; GRESSLER et al., 2012). In United States, was discovered the highly virulent *C. jejune* clone (clone SA, for sheep abortion) as the predominant cause of Campylobacter-associated abortion in sheep, all isolates were resistant to tetracycline, is quite intriguing and suggests that the clone is ecologically well adapted and pathologically hypervirulent in ruminants (SAHIN et al., 2008).

This difference between the association of clinical signs may occur due to the variability of strains of *Campylobacter jejuni* and *Campylobacter coli* (COLLINS; LISLE, 1984; DELONG et al., 1996) or due to the inability to isolate *Campylobacter fetus fetus* from the 255 fecal samples from ewes with reproductive disorders, given that *Campylobacter fetus fetus* is frequently associated with abortion (DENNIS, 1975; BIRD et al., 1984; COLLINS; LISLE, 1984; KIRKBRIDE, 1993; DELONG et al., 1996; FENWICK et al., 2000; MANNERING et al., 2004; AGERHOLM et al., 2006; SAHIN et al., 2008). Another characteristic of the isolated

species is that they can be found in both healthy animals (STANLEY et al., 1998; JONES et al., 1999; AÇIK; ÇETINKAY, 2006; OPORTO et al., 2007; SPROSTON et al., 2010) and animals with reproductive disorders (DELONG et al., 1996; VARGAS et al., 2005; SAHIN et al., 2008).

In the case of placental retention (p=0.032 and a risk factor of 15.36), no association was observed with infection as in other studies; however, of the three animals that had placental retention, one was positive for *Campylobacter* spp.

Among the clinical signs observed during the clinical exam, a significant relationship was observed between animals with diarrhea and isolates of *Campylobacter* spp. (p= 0.003) with a risk factor of 9.83, in agreement with Walt (1994) who reported cases of diarrhea before episodes of abortion. However, among the three females with diarrhea who were positive for *Campylobacter* spp., only one had a history of abortion, and the other two gave birth to "weak" lambs that may have been contaminated immediately after delivery.

Conclusion

Note that the dissemination of *Campylobacter* spp. in Brazilian sheep is low and that, at present, the existing strains are not responsible for significant economic losses in sheep production, especially in adult animals. However, there is a risk that these bacteria can lead to the birth of "weak" lambs and subsequent neonatal death, and of the spread of bacteria in the herd through cases of diarrhea, especially during periods in which animals are under stress, predisposing lambs to fecal-oral infection and compromising their development.

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