



Research article

Incidence of *Escherichia coli* O157:H7 in feces of lactating ruminants with diarrheal syndrome

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ABSTRACT

Objective. To identify *Escherichia coli* O157:H7 present in diarrheal feces of lactating ruminants with diarrheal syndrome and safety of colostrum intake. **Materials and methods.** A feces sampling of 316 ruminants was carried out during the period of August 2015 to March 2016 in the municipalities of Río Grande, General Enrique Estrada, Morelos and Calera de Victor Rosales of the state of Zacatecas, obtained from 67 cattle, 183 sheep and 66 goats. **Results.** The following were identified in CHROMagar™ chromogenic medium: 260 coliforms, 78 *Escherichia coli* O157:H7, 16 *Proteus* spp. and 25 colonies of unidentified bacteria, finding an incidence of *Escherichia coli* O157:H7 of 22.03% in the four municipalities. **Conclusions.** *Escherichia coli* O157:H7 is the second bacteria found in ruminant feces with an incidence of 22%, which is a mortality risk factor in lactating ruminants (less than 21 days old), causing economic loss and health risk for the population of the state of Zacatecas.

Keywords: Bacteria, chromogenic medium, sheep (*Source: CAB*).

RESUMEN

Objetivo. Identificar *Escherichia coli* O157:H7 presente en heces diarreicas de rumiantes lactantes con síndrome diarreico y seguridad de ingesta de calostro. **Materiales y métodos.** Se realizó un muestreo de 316 rumiantes durante el período de agosto 2015 a marzo 2016 en los municipios de Río Grande, General Enrique Estrada, Morelos y Calera de Víctor Rosales del estado de Zacatecas, 67 de bovinos, 183 de ovinos y 66 de caprinos. **Resultados.** Se identificaron en medio cromogénico CHROMagar™: 260 coliformes, 78 *Escherichia coli* O157:H7, 16 *Proteus* spp., y 25 colonias de bacterias sin identificar con este medio, encontrándose una incidencia de *Escherichia coli* O157:H7 de 22.03% en los cuatro municipios. **Conclusiones.** *Escherichia coli* O157:H7 es la segunda bacteria encontrada en heces de rumiantes con un 22% de incidencia, la cual es un factor de riesgo de muerte en rumiantes lactantes (menos de 21 días de nacidos) causando pérdidas económicas y riesgo para la salud de la población del estado de Zacatecas.

Palabras clave: Bacterias, medio cromogénico, ovinos (*Fuente: CAB*).

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INTRODUCTION

The economic repercussion resulting from digestive diseases in ruminants in Mexico is serious and frequent. The pathological symptoms presented by the ruminants under this type of stress are as follows: low feed consumption, presence of diarrheas (or dysentery), ruminal acidosis, laminitis, limp, delay in corporal development of lambs, kids and calves (1). This means an unforeseen added cost for the producer, along with expenses in veterinary treatments (2).

In intensive production systems, the mortality index of lambs due to digestive diseases prior to weaning tends to be less than 10%, whereas in extensive systems the losses can be as high as 53% of the lambs born (3). However, various reports mention that infectious processes and nutritional and metabolic disorders represent the principal cause of neonatal mortality (4). In fact, between 15 and 20% of the neonatal losses can be attributed to digestive diseases, and consequently, the rest (around 80%) are the result of environmental or management factors (3).

The Centers for Disease Control (CDC) of the Department of Health and Human Services of the United States mention that *Escherichia coli* is a type of bacteria that normally lives in the intestines of humans and animals. For the most part they are inoffensive and in fact are part of a healthy intestinal tract. However, some serotypes are pathogens, causing diarrheas or diseases outside of the intestinal tract (5).

Escherichia coli is classified into six serotypes that are associated with diarrhea; among these is enterohemorrhagic *Escherichia coli* (EHEC), which causes hemorrhagic colitis (HC) and is identified through the production of Shiga toxins (Stxs). Shiga toxin-producing *Escherichia coli* (STEC) is a group of zoonotic pathogens, and *Escherichia coli* 0157:H7 is the serotype associated with hemorrhagic colitis and hemolytic uremic syndrome (HUS), principally in the United States.

Laven et al (7) demonstrated that the presence of *Escherichia coli* in ruminants was higher in the colon than in the rumen, associating the presence of *Escherichia coli* to the ingestion phase and not to the intestinal wall of the rumen. Studies have demonstrated that the terminal part of the colon is the principal development site of the colonization of *Escherichia coli* 0157:H7 and that the lymphoid tissue located in the recto-anal junction is the colonization site in cattle (8). Therefore, this colonization is considered a "super-propagator" which causes severe infections with epidemiological manifestations characterized by hemorrhagic diarrheas, colitis and hemolytic uremic syndrome (9).

Adequate isolation and identification methods are crucial for the diagnosis of *Escherichia coli* 0157:H7. Before confirming its presence in a sample, it is necessary to isolate and identify it in selective and differential culture media. The media used conventionally for the isolation of *Escherichia coli* 0157:H7 include Sorbitol MacConkey Agar (SMAC), CefiximeTellurite Sorbitol MacConkey Agar (CT-SMAC), CHROMagar™ 0157 (CHROMagar), Tellurite CHROMagar™ 0157 (T-CHROMagar) and Vancomycin Cefixime Cefsoludin CHROMagar™ 0157 (VCCCHROMagar) (6,10).

The chromogenic chemical substrates patented for the identification of microbial colonies led to the development of a wide range of media for the detection of pathogens. The medium CHROMagar™ 0157 selectively promotes the growth of *Escherichia coli* 0157:H7 and the difference from *Escherichia coli* no. 0157, due to a chromogenic substrate of β -galactosidase and a change in the pH (6). This medium is used for the detection of *Escherichia coli* 0157:H7 in alimentary as well as environmental samples, but could be more selective with the addition of potassium tellurite, or with vancomycin, cefixime and cefsoludin (11).

Therefore, the objective of the present study was to identify *Escherichia coli* 0157:H7 in diarrheic feces of lactating ruminants younger than 21 days through the use of agar CHROMagar™, which will permit producers to make decisions about which treatments to employ in order to avoid the incidence of this pathogen and reduce neonatal mortality.

MATERIALS AND METHODS

Samples. 316 swabbed rectal samples from 183 sheep, 66 of goats and 67 of cattle under 21 days of age were obtained, and with the security of having ingested colostrum, with presence of diarrheic syndrome. The samples were collected rectally with a sterile cotton swab, and were labeled and transported in Stuar® medium. The sampling was carried out during the period of August 2015 to March 2016 in four municipalities of the state of Zacatecas: in Calera de Victor Rosales, the localities El Bordo, Noria de Agostadero, El Duraznillo, El Vergel and El Porvenir: in Morelos the localities of Hacienda Nueva, Noria de Gringos, Las Pilas and El Palmar: in General Enrique Estrada, Los Ángeles, Puenteclillos and San Sebastián, and in Río Grande the localities El Carrizal, Los Ramírez, San José de Ranchitos and Tierra Blanca (Figure 1). (Coordinates: El Bordo 102°49'20"O 22°57'02"N, San José de Ranchitos 102°57'35"O 23°40'0"N, El Carrizal 103°01'08"O 23°38'25"N,

El Duraznito 102°38'26"O 23°01'29"N, El Palmar 102°39'58"O 22°53'24"N, El Porvenir 102°40'00"O 22°56'42"N, El Vergel 102°41'01"O 22°59'58"N, General Enrique Estrada 102°44'29"O 22°59'40"N, Hacienda Nueva 102°36'21"O 22°49'30"N, Las Pilas 102°36'46"O 22°50'24"N, Los Ramírez 103°01'52"O 23°50'49"N, Los Ángeles 102°43'22"O 22°58'43"N, Noria De Agostadero 102°46'59"O 22°53'14"N, Noria de los Gringos 102°43'04"O 22°50'53"N, PuenteCillos 102°45'03"O 23°02'47"N, San Sebastián 102°47'54"O 23°03'37"N, Tierra Blanca 103°04'36"O 23°49'38"N).

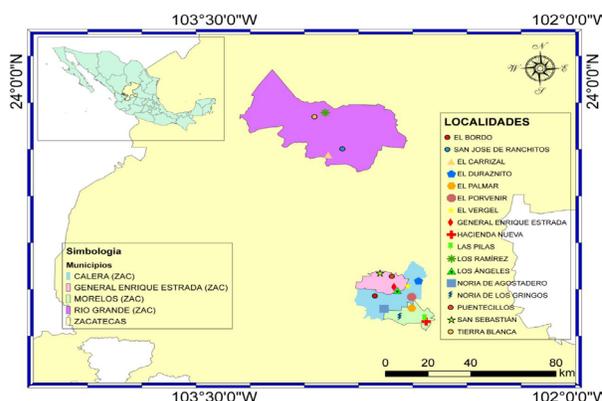


Figure 1. Geographic location of the localities sampled in the four municipalities of the state of Zacatecas.

Gram negative bacterial culture. The cultures were made in duplicate. Each sample was sown in Petri jars with MacConkey agar (SMAC), and incubated in a bacteriological oven at 37 °C for 48 hours (12). The creamy pink colonies were considered positive and were individually isolated for their later re-sowing.

Identification of bacteria in chromogenic medium CHROMagar™. After the bacteria were isolated in MacConkey agar, a colony was taken for the identification of the serotype of *Escherichia coli* O157:H7 or its non-mobile variant O157:H (serotype VTEC), which is more common in public health. The re-sowing was made by plate striation in chromogenic medium CHROMagar™ and was incubated at 37 °C during 24 hours (13) (Table 1).

The colonies of *Escherichia coli* O157:H7 grow in CHROMagar™ O157 and produce a mauve pink color due to chromogenic substrates in the medium, thus permitting the presumptive identification of the primary isolation plate and the differentiation from other organisms (13) (Table 1).

Table 1. Characteristics of the colonies using the CHROMagar™ chromogenic medium.

Microorganism	Aspect of the colony	Observation in culture
<i>E. coli</i> O157:H7	mauve pink	
Coliformes	metallic blue	
<i>Proteus</i>	colorless to gray	

Statistical analysis. The experimental data were analyzed first with descriptive statistics, and afterwards using a classification table or contingency table. The Chi-squared statistical analysis was used, which is a statistic that makes it possible to determine the degree of independence among the variables. The assumption of this test is as follows: H_0 : The prevalence of the bacteria is independent from the type of ruminant the carrier may be; H_a : The prevalence of the bacteria is dependent on the type of ruminant carrier. The most important index of this test is the value p . In general, when the value p is less than 0.05, H_0 is rejected and it is concluded that the bacteria are not independent. All of the statistical process was carried out with SPSS 25.0 for Microsoft Windows.

RESULTS

The determination of *Escherichia coli* O157:H7 was made using chromogenic medium CHROMagar™ differentiating the bacterial colonies by color; *Escherichia coli* O157:H7 presented mauve pink coloration, *Proteus* spp. are from colorless to gray and the coliforms present metallic blue colored colonies, as can be seen in Figure 2.

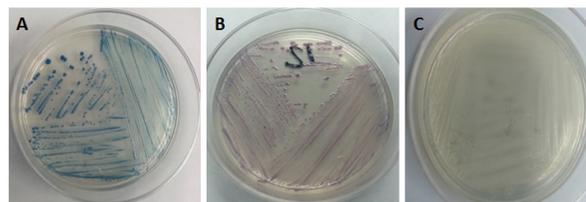


Figure 2. Development of bacteria in CHROMagar™. A) Coliforms, B) *E. coli* O157:H7 and C) *Proteus* spp.

Some samples presented development of two or more colonies with distinct morphology and coloration, thus the total number of the bacterial colonies identified was 354, and in 25 cases there was no development in 24 hours, despite the fact that development was found in MacConkey agar and colonies with descriptive morphology for *Escherichia coli* there was no development in CHROMagar™.

260 coliforms were identified, along with 78 *Escherichia coli* O157:H7 and 16 *Proteus* spp., in all of the ruminants sampled, of which 183 were sheep, where it was found that in 46 sheep there was presence of *Escherichia coli* O157:H7, in goats it was found in 7 of 66 and in cattle, 25 of 67 as is shown in Table 2. Furthermore, using the chi-squared test it is confirmed that the prevalence of the bacteria (coliforms, *Escherichia coli* O157:H7 and *Proteus* spp.) depends on the type of ruminant (sheep, cattle and goat) which is the carrier of the bacteria (Table 3).

Table 2. Number of bacteria obtained from sowing in CHROMagar™.

Source	# Animals	Coliforms	<i>E. coli</i> O157:H7	<i>Proteus</i> spp.	N.D.	# Bacteria
Sheep	183	153	46	8	14	221
Goats	66	61	7	4	4	82
Bovine	67	46	25	4	7	76
Total	316	260	78	16	25	379

N.D.: Not determined

Table 3. Contingency table: type of bacteria vs ruminant type.

		Type of ruminant			Total
		Sheep	Bovine	Goats	
Prevalence of the bacteria	<i>E. coli</i> O157:H7	46	25	7	78
		20.8%	30.5%	9.2%	20.6%
	Coliforms	153	46	61	260
		69.2%	56.1%	80.3%	68.6%
	<i>Proteus</i>	8	4	4	16
		3.6%	4.9%	5.3%	4.2%
	N.D.	14	7	4	25
		6.3%	8.5%	5.3%	6.6%
Total		221	82	76	379
		100.0%	100.0%	100.0%	100.0%

H₀: The prevalence of bacteria is independent of the type of ruminant that is the carrier; H₁: The prevalence of bacteria is dependent on the type of ruminant that is the carrier. Chi-square value of Pearson=13.243. *gl*=6; *p*=0.039. As the value of *p*≤0.05 is rejected H₀. N.D.: No Determinados.

The distribution of bacteria found according to their geographic location correspond to the following: 18 *Escherichia coli* O157:H7, 60 coliforms and 7 *Proteus* spp., in the municipality of Calera de Victor Rosales; 40 *Escherichia coli* O157:H7, 11 coliforms and 9 *Proteus* spp., in localities of Morelos, 24 *Escherichia coli* O157:H7, 41 coliforms and 1 *Proteus* spp., in localities General Enrique Estrada and 16 *Escherichia coli* O157:H7, 99 coliforms and 4 *Proteus* spp., in localities of the municipality of Río Grande (Table 4).

Table 4. Bacteria determined by location and type of ruminant.

Municipalities	Localities	<i>E. coli</i> O157:H7			Coliforms			<i>Proteus</i>		
		Sheep	Bovine	Goats	Sheep	Bovine	Goats	Sheep	Bovine	Goats
Calera de Víctor Rosales	El bordo	1	0	0	9	1	2	1	0	0
	Noria de agostadero	1	0	0	12	2	2	0	0	1
	El duraznillo	5	4	1	5	1	1	1	0	0
	El porvenir	5	0	0	11	2	3	0	1	1
	El vergel	1	0	0	6	2	1	1	0	1
Morelos	Hacienda Nueva	2	0	0	3	3	3	0	1	0
	Noria de gringos	3	0	2	20	5	1	0	1	0
	Las pilas	2	1	0	4	1	4	1	0	0
	El palmar	2	8	0	13	2	1	1	0	0
General Enrique Estrada	General Enrique Estrada	3	0	0	3	2	3	0	0	0
	Los Ángeles	5	0	0	2	1	3	0	0	0
	Puentecillos	8	0	0	5	0	6	1	0	0
	San Sebastián	4	3	1	8	5	3	0	0	0
Río Grande	El carrizal	0	0	0	8	4	6	1	0	0
	Los Ramírez	4	0	3	9	3	7	1	0	0
	San José de Ranchitos	0	5	0	16	7	7	0	1	1
	Tierra blanca	0	4	0	19	5	8	0	0	0
Total:		46	25	7	153	46	61	8	4	4

The highest incidence of bacteria were the coliforms with 73.45% in the four municipalities, followed by *Escherichia coli* O157:H7 with 22.03% and of *Proteus* with 4.52% (Table 5). With these results, the producers of the region are able to make the best decision of prevention treatments to reduce the incidence of this pathogen and thus reduce mortality in lactating ruminants.

Table 5. Percentage of bacteria incidence by municipality and type of animal.

Municipalities	<i>E. coli</i> O157:H7			Coliforms			<i>Proteus</i>		
	Sheep	Bovine	Goats	Sheep	Bovine	Goats	Sheep	Bovine	Goats
Calera de Víctor Rosales	3.67	1.13	0.28	12.15	2.26	2.54	0.85	0.28	0.85
Morelos	2.54	2.54	0.56	11.30	3.11	2.54	0.56	0.56	0.00
General Enrique Estrada	5.65	0.85	0.28	5.08	2.26	4.24	0.28	0.00	0.00
Río Grande	1.13	2.54	0.85	14.69	5.37	7.91	0.56	0.28	0.28
Incidence %	22.03%			73.45%			4.52%		

DISCUSSION

The presence of *Escherichia coli* 0157:H7 in cattle should be taken into consideration as a possible problem of public health, given that this bacteria strain is present in feces and is a source of infection for humans. In the finishing process of the animal, refrigeration of the carcass and also in milking, contamination of the meat and milk may occur (14).

It has been reported that the presence of *Escherichia coli* 0157:H7 in cattle feces seems to be influenced by the age of the animal (15). For example, calves under eight weeks of age (56 days) and heifers excrete more of the *Escherichia coli* strain than adult cattle (16).

Escherichia coli 0157:H7 has become an important problem for human health, principally in the United States (6); Olvera et al (16) have stated that cattle are the primary reservoir of the bacteria *Escherichia coli* 0157:H7, which causes hemolytic uremic syndrome in humans. In fact, the carcasses from feedlot systems present the highest incidence of verotoxigenic *Escherichia coli* associated with hemolytic uremic syndrome, with respect to the grazing system where vaccination reduced the contamination of these bacteria in beef by 54.1%.

To diagnose this pathogen, adequate isolation and identification methods are crucial. There is little information available on its prevalence in cow/calf management. However, in Louisiana, U.S., an 8% prevalence of this pathogen has been reported, without significant differences among fecal matter, water (from water troughs and ponds) and surface smears (of water troughs and hay bunk) as sources of contamination (6).

In the investigation conducted by Fox et al (18), a general prevalence of 62% was found, higher than what was obtained in the present study (22.03%). The prevalence of *Escherichia coli* 0157:H7 in cattle

is variable among each individual and each herd. In stabled cattle, it can be found in a range of 10 to 28%, but it can be higher, as much as 80% in the summer months (19). Cattle are the most affected, followed by sheep (20), which indicates there is no host selection for *Escherichia coli* 0157:H7.

With the use of CHROMagar™ it has been possible to rapidly determine the incidence with an identification sensitivity of 98% and a specificity of 100% (13), being superior to MacConkey sorbitol agar, which despite having the same sensitivity, its specificity decreases to 85% and has a precision of 86%. This medium was initially used only in the food industry for the rapid release of pathogen free foods, but it has been tested for clinical sample analyses and has been used in various studies (6, 14, 21, 22). However, for precision of the method, investigation should be continued, focusing on the characterization based on the sequence of nucleic acids through PCR procedures (polymerase chain reaction) that point to genes of the shiga toxin (stx1 and stx2), given that BBL CHROMagar 0157 does not differentiate between the toxin producing and non-toxin producing strains of *Escherichia coli* 0157:H7 (13).

In conclusion, the coliform bacteria had the highest presence in the samples analyzed. However, they are not considered to be the causal agent of diarrhea. The presence of *Escherichia coli* 0157:H7 was the second bacteria with highest percentage in the fecal samples analyzed (22.03% incidence), which does not depend on the species of the ruminant, but it represents a mortality risk factor in lactating ruminants (under 21 days of age) causing economic losses and health risk for the population of the state of Zacatecas.

Conflict of interests.

The authors declare no conflict of interest with publication of this manuscript.

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