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## Ruminal lesions in cattle slaughtered in slaughterhouses

Lesiones ruminales en bovinos sacrificados en rastro

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### ABSTRACT

The objective of the present study was to describe macroscopic and microscopic lesion in the rumen of bovine slaughtered in the Municipal Meat Processor of Colima, Mexico. The lesions found during the slaughter were macroscopically and subsequently fixed in 10% buffered formalin with pH 7.2 described. It was with the routine histological technique, and stained with hematoxylin-eosin (H-E) processed. 100 rumen were collected, as well as data on the origin, age, sex, and species of the animals. Data were, with the Chi-square independence test and logistic regression analysis analyzed. The 98% of the bovines presented at least one of the following lesions: hemorrhages, erosions, ulcers, scars, hyperkeratosis, hydropic degeneration, rumenitis, lymphangiectasia, a sample had a papilloma and the presence of the protozoan *Balantidium coli* was in 30% of the samples found. Dependence was found between *B. coli* to the origin ( $P=0.046$ ) and the sex ( $P=0.041$ ) of the animals. The variables that were significant ( $P<0.05$ ) in the logistic regression analysis were scars, rumenitis, lymphangiectasia, and *B. coli*. The injuries found to interfere with the absorption of nutrients, loss of production, and productivity, as well as the presence of opportunistic pathogens that can represent a health risk for animals and humans.

**Keywords:** hyperkeratosis, rumenitis, *Balantidium coli*, ruminal acidosis.

### RESUMEN

El objetivo del presente estudio fue describir los hallazgos macroscópicos y microscópicos encontrados en el rumen de bovinos sacrificados en la Procesadora Municipal de Carne de Colima, Colima, México. Las lesiones encontradas durante la faena se describieron macroscópicamente, posteriormente se fijaron en formalina amortiguada al 10% con pH 7.2, se procesaron con la técnica histológica de rutina y se tiñeron con hematoxilina-eosina (H-E). Se colectaron 100 rúmenes, así como datos de la procedencia, edad, sexo y especie de los animales. La información fue analizada con la prueba de independencia de Chi cuadrada y un análisis de regresión logística. El 98% de los bovinos presentaron al menos una de las siguientes lesiones: hemorragias, erosiones, úlceras, cicatrices, hiperqueratosis, degeneración hidrópica, rumenitis, linfangiectasia, una muestra presentó un papiloma y se encontró la presencia del protozoario *Balantidium coli* en el 30% de las muestras. Se encontró dependencia entre *B. coli* con la procedencia ( $P=0.046$ ) y el sexo ( $P=0.041$ ) de los animales. Las variables que fueron significativas ( $P<0.05$ ) en el análisis de regresión logística fueron las cicatrices, rumenitis, linfangiectasia y *B. coli*. Las lesiones encontradas

interfieren con la absorción de nutrientes, pérdida de producción y productividad, así como la presencia de patógenos oportunistas que pueden representar un riesgo sanitario para animales y humanos.

**Palabras clave:** hiperqueratosis, rumenitis, *Balantidium coli* y acidosis ruminal.

## INTRODUCTION

There are many diseases or digestive system disorders in cattle. They are of economic importance in the production units, since they cause a decrease in production, high morbidity and mortality; they reduce animal welfare, as well as an impact on public health (Plazier *et al.*, 2008). The rumen carries out a series of adaptations to the different feeding phases, to which the bovines are subjected, in which forages and large quantities of grains are digested; which can cause the appearance of digestive disorders, such as acidosis, resulting from the rapid fermentation of the grain (Meyer y Bryant, 2017). Ruminal acidosis (RA) problems are common. However, it is a difficult disorder to recognize and prevent, since the pathophysiology is complex and variable (Oetzel, 2017). This disease is caused by eating high concentrate diets; it is defined as a decrease in pH (<5.6), for prolonged periods of up to at least three hours per day (Kleen *et al.*, 2003; Gozho *et al.*, 2005; Krause y Oetzel, 2006; Steele *et al.*, 2009).

Low pH induces lysis of Gram-negative bacteria, and increases free lipopolysaccharide (LPS) in the rumen, which is a powerful inducer of inflammation and the development of rumenitis (Zhao *et al.*, 2018). The production of immunogens in the rumen, such as LPS or histamine, cause the rumen protection barrier to be reduced and they are implicated in the development of this disease (Plazier *et al.*, 2008).

Ruminal pH is a key factor for the correct functioning of the rumen, since it affects microbial populations, products of fermentation and its physiological functions (Nagaraja y Titgemeyer, 2007). A decrease in: fat in milk, reduced fiber digestion, loss of body condition, diarrhea, laminitis, inflammation and tympanism can be observed; in addition to affecting the voluntary consumption of food (Panciera *et al.*, 2007; Rezac *et al.*, 2014a; Zhao *et al.*, 2018).

These alterations can cause an imbalance in the ruminal microbiota, where etiological agents, such as *Fusobacterium necrophorum*, *Trueperella pyogenes* and *Arcanobacterium* spp, among others, behave like opportunists, and travel through the portal circulation to the liver, where they can generate abscesses (Tadepalli *et al.*, 2009; Xu y Ding, 2011; Trigo, 2015). Alteration of the ruminal epithelium allows microorganisms and their toxins to migrate into the portal circulation, which can predispose to a variety of infections or endotoxins in the bloodstream, as they alter the ability of nutrient absorption; which can generate an accumulation of volatile fatty acids (AGV) or lactic acid (Kleen *et al.*, 2003). The ruminal papillae are usually covered by mucus compared to the abomasal papillae not covered, so they may be more susceptible to rumenitis that can progress to erosion and ulceration (Snyder y Credille, 2017).

Parakeratosis is the thickening of the stratum corneum of the ruminal epithelium, and it is the result of chronic rumenitis (Jubb *et al.*, 2016), being an important pathology; since it decreases the absorption capacity of AGV and predisposes affected animals to new episodes (Oetzel, 2017). In Mexico, the economic impact produced by ruminal acidosis has been difficult to quantify, but it is important due to the injuries it causes in fattening cattle (Malafaia *et al.*, 2016).

There are few studies in Mexico that describe the injuries observed in the rumen at the time of the slaughter of the animals, the rumen inspection is a tool that allows evaluating the nutritional health of livestock in the pens. Therefore, the objective of the present study was to describe the ruminal lesions present in cattle that arrive at the municipal meat processor in Colima city, Mexico.

## MATERIAL AND METHODS

### Study area

The work was in Colima state carried out, at the municipal meat processor, located at km. 3.5 of the Colima-Coquimatlán highway. The predominant climate in 78.52% of the territory is warm sub-humid with rains in summer, in 12.42% there is a semi-dry warm climate, in 7.58% semi-warm sub-humid with rains in summer and in 1.48% of the territory, a temperate sub-humid climate with rains in summer (INEGI, 2017). The average annual temperature is 25 °C and the total annual precipitation is approximately 1728.9 mm (CONAGUA, 2017).

### Sampling

100 samples of ruminal tissue were collected, 1 cm<sup>2</sup> from the right side of the cranio-ventral sac and from the left side of the cranial ventral sac, because in this ruminal portion the epithelium is in longer contact with ruminal fluid (Castro y Elizondo, 2012; Jonsson *et al.*, 2019). Some representative areas, where some type of macroscopic injury has been observed; which were fixed by immersion in 10% buffered formalin for 24 h. The tissues were in plastic cassettes, cut and included. They were transferred to the Pathology Laboratory of the Faculty of Veterinary Medicine and Animal Husbandry of the University of Colima, located in Tecomán municipality, Colima, Mexico at km. 40, Colima-Manzanillo highway, within the geographic coordinates 18° 55' of North Latitude and 103° 52' of West Longitude, and an altitude of 20 m a.s.l (INEGI, 2017). Subsequently, they were processed with the routine histological technique, which consists of dehydration, clearance and paraffin infiltration; they were cut at 5 µm thick and stained with Hematoxylin-Eosin (H-E) staining (Prophet *et al.*, 1995).

## Sample analysis

Macroscopic and microscopic lesions were using pathological concepts described and characterized; the results were expressed in percentages and degrees of severity (Rezaca *et al.*, 2014b) Likewise, the Chi square test was used to assess the dependency between the lesions with the place of origin of the animals, age, species and sex. A logistic regression analysis was performed in order to find risk factors associated with the presence of injuries. Both analyzes were performed with the Statgraphics Centurion ver.15.2 program (Statgraphics, 2007).

For the statistical analysis, two age ranges were established, less than or equal to 36 months, and greater than or equal to 37 months; trying to maintain an adequate number of records at each level of classification. For the species, the Angus, Beefmaster, Brown Swiss and Simmental racial genotypes were classified in *Bos Taurus* and zebu were classified in *Bos indicus*. The places of origin were into four regions grouped, according to the type of climate: region 1: temperate sub-humid, region 2: semi-warm sub-humid, region 3: warm sub-humid and region 4: warm.

## RESULTS

From the total samples, 33 were females and 67 males. Of the slaughtered animals, 56 belonged to the *Bos taurus* species and 44 to *Bos indicus*. The 98% of the samples presented at least one lesion. The macroscopic findings were (figure 1): hemorrhages, erosion, ulcers, hyperkeratosis (thickening of the rumen papillae), atrophy of the papillae and scars; one sample had a papilloma. The microscopic findings (figure 2) were thickening of the horny layer of the ruminal papillae, as well as the presence of collagen fibers, hydropic degeneration, inflammation (rumenitis) and lymphangiectasia. In addition, the presence of the protozoan *Balantidium coli* was recorded in 30% of the samples. These injuries were grouped into degrees of severity, which ranged from mild to severe (Table 1).

**Table 1. Number of cases for rumen injuries and their classification in degrees of severity**

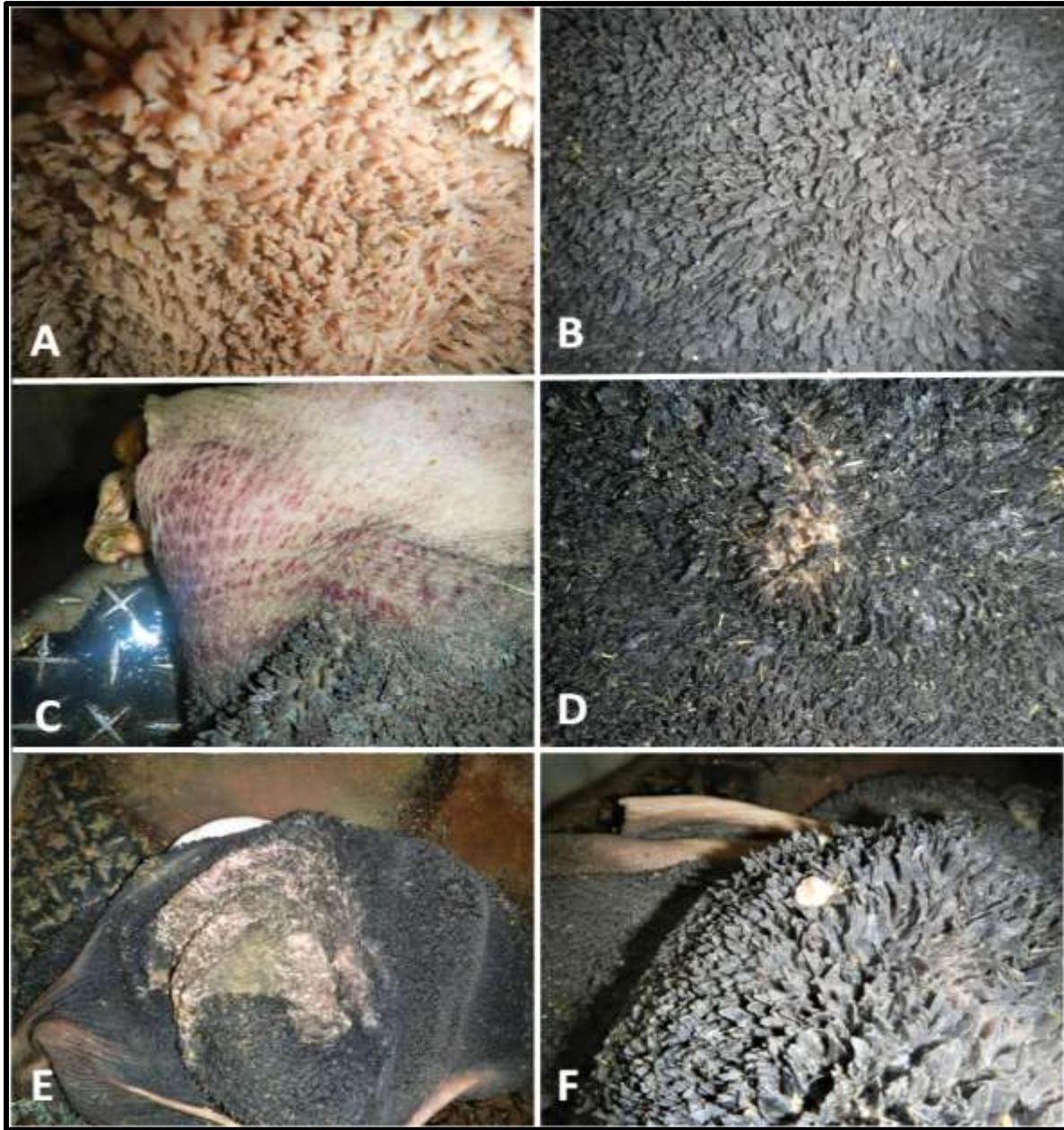
Degree of severity	Hyperkeratosis	Hydropic degeneration	Rumenitis	Lymphangiectasia	<i>Balantidium coli</i>
Slight	33	53	23	21	20
Moderate	42	30	18	6	6
Severe	18	9	1	1	4
Total case	93	92	42	28	30
No apparent injury	7	8	58	72	70

**Table 2. Risk factors for the presence of ruminal lesions in cattle slaughtered at the Municipal Meat Processor in Colima. Mexico**

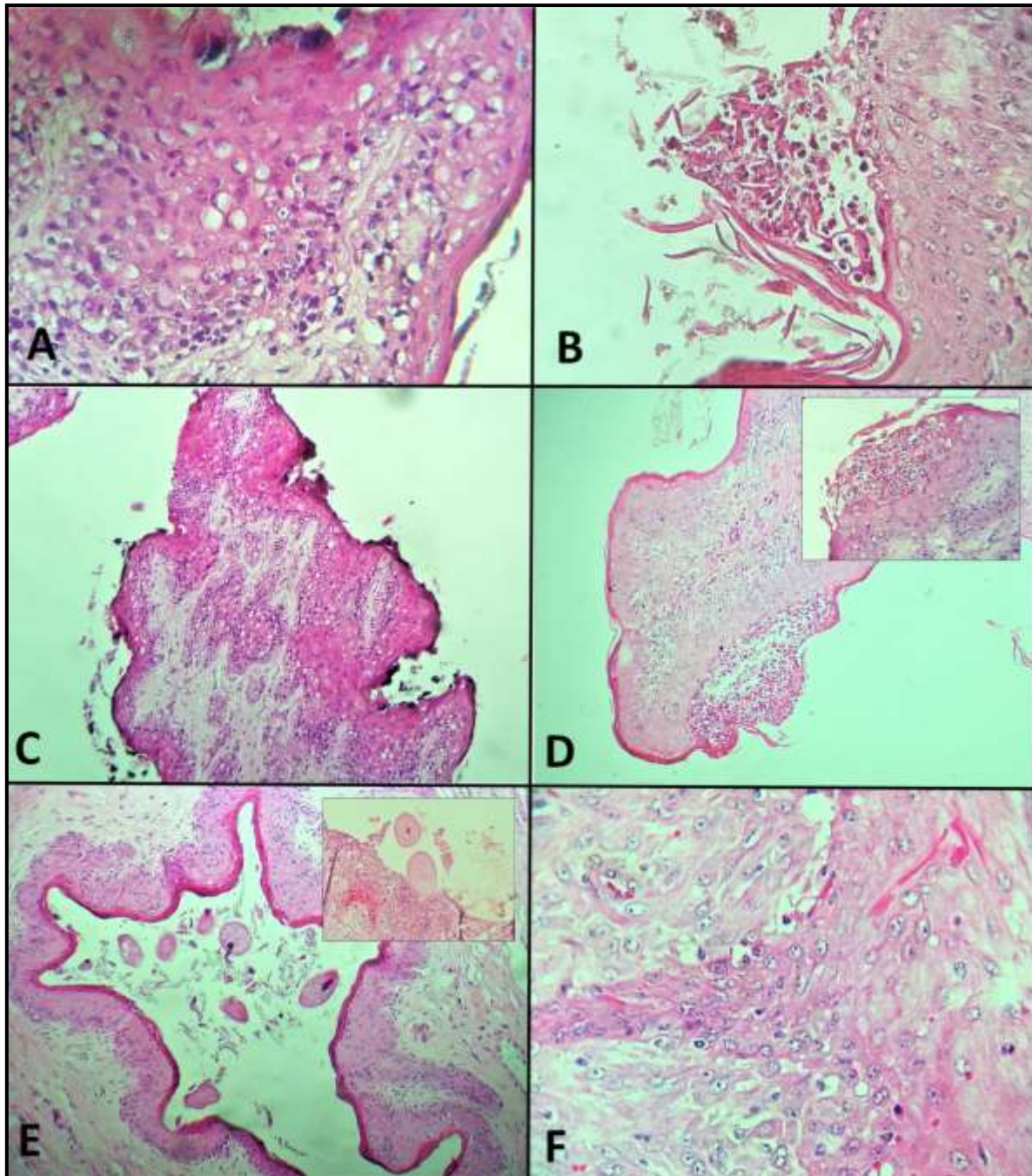
Variables	Estimate	Standard error	Odd Ratio	IC(OR) 95%		Value of P	
				Superior limit (LI)	Inferior limit (LS)		
Scar							
<b>Provenance</b>	Region 1	-0.230	0.067	0.793	0.694	0.907	0.000
	Region 2	-0.685	0.05	0.503	0.455	0.556	
	Region 3	0.402	0.089	1.495	1.250	1.787	
<b>Age</b>		-0.142	0.045	0.866	0.791	0.949	0.000
<b>Species</b>		-0.515	0.046	0.597	0.545	0.654	0.000
<b>Sex</b>		-0.299	0.049	0.741	0.672	0.817	0.000
Rumenitis							
<b>Provenance</b>	Region 1	0.949	0.0711	2.583	2.243	2.975	0.000
	Region 2	0.176	0.049	1.192	1.080	1.317	
	Region 3	-1.001	0.080	0.367	0.313	0.430	
<b>Age</b>		0.310	0.045	1.364	1.246	1.494	0.000
<b>Species</b>		0.725	0.044	2.066	1.891	2.258	0.000
<b>Sex</b>		-0.092	0.048	0.911	0.828	1.003	0.000
Lymphagiectasia							
<b>Provenance</b>	Region 1	-1.177	0.067	0.308	0.269	0.352	0.000
	Region 2	-0.066	0.056	0.935	0.836	1.046	
	Region 3	-0.431	0.083	0.649	0.55	0.765	
<b>Age</b>		-0.448	0.049	0.638	0.578	0.704	0.000
<b>Species</b>		-0.266	0.05	0.766	0.693	0.846	0.000
<b>Sex</b>		0.453	0.056	1.573	1.406	1.759	0.000
<i>Balantidium coli</i>							
<b>Provenance</b>	Region 1	-1.211	0.074	0.297	0.256	0.344	0.000
	Region 2	-1.229	0.06	0.292	0.259	0.329	
	Region 3	-1.657	0.085	0.19	0.16	0.225	
<b>Age</b>		-0.477	0.05	0.62	0.56	0.686	0.000
<b>Species</b>		0.501	0.048	1.65	1.499	1.816	0.000
<b>Sex</b>		-0.779	0.052	0.458	0.413	0.5	0.000

( $P < 0.05$ )

4 was, as a comparison for the provenance variable taken: warm climate; for the age the bovines smaller than 36 months for the sex the females and for the *Bos taurus* species.



**Figure 1. Macroscopic description of ruminal lesions.** A/ normal rumen mucosa. B/ thickening and atrophy of the rumen papillae. C/ ruminal hemorrhages. D/E erosions, ulcers and ruminal scars. F/ presence of 1X2 cm wart or papilloma showing a cauliflower-like mass, white in color, growing above the epithelium on the surface of the ruminal mucosa and covered with scales.



**Figure 2. Microscopic description of ruminal lesions.** A/ Thickening of the horny layer of the ruminal papillae, as well as the presence of epithelial hydropic degeneration. Hematoxylin-eosin 40X staining. B-C/ Erosion, ulcer and parakeratosis of the ruminal papilla. Hematoxylin-eosin 40X staining. D/ Mononuclear inflammatory infiltrate over the submucosa of the ruminal papilla (rumenitis), inset with zoom. Hematoxylin-eosin 40X staining. E/Presence of abundant protozoa *Balantidium coli* on the mucosa and ruminal epithelium causing hemorrhages and inflammation (box). Hematoxylin-eosin staining 10-40X. F/Epithelial hyperplasia of the ruminal papillae with cytoplasmic vacuolization and hyperkeratosis, in addition to proliferation of collagen, forming digital projections towards the submucosa (papilloma). Hematoxylin-eosin 40X staining.

According to the frequencies found, bovines from sub-humid semi-warm climates, whose species was *Bos indicus*, older than or equal to 37 months and males, presented more injuries. The presence of the protozoan *B. coli* was significant in the Chi square test, showing dependence with the origin ( $P=0.046$ ) and sex ( $P=0.041$ ) of the animals. In the logistic regression analysis the variables: origin, age, species and sex of the animals, were significant ( $P=0.000$ ) for the presence of scars, rumenitis, lymphangiectasia and *B. coli* (Table 2).

Cattle from semi-humid sub-humid climates were 1.49 times more likely to present rumen scars; compared to animals from warm climates. Cattle from subhumid temperate climates were 2.5 times more likely to present rumenitis than the ones from warm climates. Cattle older than 37 months were 1.3 times more likely to present rumenitis, compared to cattle less than or equal to 36 months. The *Bos taurus* species was 2.06 times more likely to have rumenitis, compared to *Bos indicus*. Similarly, *Bos taurus* was 1.65 times more likely to present *B. coli* than the *Bos indicus* species. Male cattle were 1.57 times more likely to have lymphangiectasia, compared to females. The *Bos indicus* species was 1.65 times more likely to present *B. coli* than the *Bos taurus* species.

## DISCUSSION

The bovines used for meat production in Colima state, come from prairie or mixed fattening systems. In mixed systems, the initiation stage begins in the meadow and ends in the pens, with the challenge of fattening for approximately 100-120 days, with the known “hot diets” consisting of the administration of large amounts of concentrate and the consequent decrease in rumen pH. It causes the appearance of rumen lesions; figure that in the present study was higher than those found by [Rezac et al. \(2014a\)](#).

In a study carried out in the United States on serious pathological conditions in cattle at slaughter, ruminal lesions had a higher frequency (35%) in 1461 cattle, the rumenitis symptoms with a decrease in the papillae was the most common lesion. The presence of scars and areas without papillae indicates a long-term exposure of the epithelium to an unfavorable environment, which may explain the degree of severity of the lesions and their frequency in adult animals. [Steele et al. \(2009\)](#) observed a detachment of the stratum corneum from the ruminal papillae, after submitting a bovine to a high grain diet, an injury not observed in the present study. It coincides with [Sasikala et al. \(2018\)](#), where the *in vivo* rumen content was observed a cornified epithelium with changes of coloration and necrosis by endoscopy in 110 cattle, which detached easily showing a hemorrhagic surface. Biopsy revealed vacuolar degeneration with nuclear changes of the squamous epithelium. Diet is the main factor in bacterial structure and ruminal function, since the epithelial bacteria of the rumen bind to the epithelial tissue, so the alteration in its composition can affect function and generate lesions ([Zhang et al., 2017](#)).



Studies suggest that there is an increase in the presence of bacteria with high virulence genes, which can take advantage of these ruminal conditions to trigger an inflammatory response (Khafipour *et al.*, 2011) or modify genes involved in the growth and structure of epithelia (Steele *et al.*, 2011). The evaluation of the ruminal microbiota in the present study was not considered, but it is believed that the bacterial diversity of the rumen and its response to diet varies considerably; even among animals fed the same diet (Penner *et al.*, 2009; Chen *et al.*, 2011).

In the present study, it was observed that the *Bos taurus* species showed a greater tendency to present rumen lesions, compared to the *Bos indicus* species; the above, according to Lees *et al.* (2017) it was mentioned that the rumen adaptation process is different between animals of the *Bos taurus* and *Bos indicus* species, particularly in tropical and subtropical climates, where zebuines have a better ability to adapt to tropical climates with temperature and high humidity, compared to *Bos taurus* (Reis *et al.*, 2016). Panciera *et al.* (2007) registered severe damages, due to acidosis, such as: abomasal tympanism, marked edema, hemorrhage and emphysema in the rumen walls; where it is suggested that part of the damage was due to the presence of clostridial species, or a group of agents that are involved in these damages. The presence of foreign objects in the rumen, such as plastic bags, can cause damage to the epithelium, such as atrophy, loss of rumen papillae, erosion, ulcers and nodular formation in the rumen mucosa (Otsyina *et al.*, 2017). The presence of ciliated protozoa is associated with the structure and pathogenicity of different bacterial communities and alterations in ruminal fermentation (García *et al.*, 2017). In addition of being an opportunistic pathogen, *B. coli* is zoonotic, and it causes gastroenteric and lung problems in man (Sharma y Harding, 2003; Pérez *et al.*, 2008; Koopowitz *et al.*, 2010).

The temperature and humidity conditions of Colima state allow the viability of this protozoan in the environment, and the infection occurs through the consumption of water or food contaminated with oocytes, excreted by animals, or even infected humans (Ahmed *et al.*, 2020).

In a study conducted in Bangladesh by Paul *et al.* (2019), identified the presence of the protozoan parasite *B. coli* in 103/200 fecal samples. The highest prevalence was recorded in cattle (54.7%), compared to pigs (42%), a result higher than that found in the present study. In China, Zhang *et al.* (2019), carried out an analysis in 468 fecal samples to identify the main infectious agents found in macaques, *B. coli*, was the second most important protozoan with 70% prevalence, a result higher than that of other species. In Brazil, a study in birds in captivity showed a prevalence of 1.4% for this same parasite using the coproparasitoscopic technique, which was found in pigeons, suggesting that they may be reservoirs for other susceptible hosts (Lyra *et al.*, 2002). Acidosis is a feeding management problem. It occurs when cattle that were consuming grass, quickly change

to a high carbohydrate diet, as in most fattening; in which the addition of some buffer to the ration such as sodium bicarbonate or calcium carbonate in no more than 5% of the ration. It makes gradual changes in the transitions of the fattening stage, as well as make routine inspections of the digestive system at the time of slaughter.

## CONCLUSIONS

Carrying out this study allowed us to identify the main types of ruminal lesions in cattle that come to slaughter to the Colima municipal meat processor. Almost the totality of bovine animals sampled suffered some type of acidosis during their life, which caused lesions in the rumen wall, causing economic losses; due to the low yields in the fattening pens. Post mortem inspection in bovines is a tool that allows evaluating ruminal health, as well as promoting preventive measures for the control of subclinical ruminal acidosis. The presence of unusual agents in the rumen suggests an alteration in the microbiota of the ruminal epithelium. This is the first study where the presence of *Balantidium coli* in ruminal tissues is documented.

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