

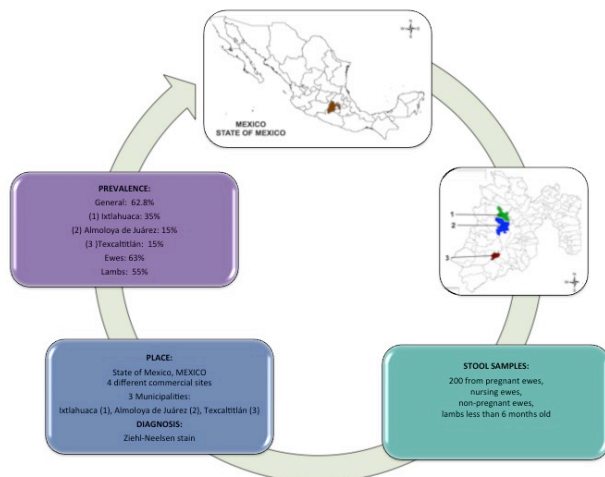
## *Cryptosporidium* spp. prevalence in sheep from commercial sites in the State of Mexico, Mexico during Summer

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### Graphical Abstract



### Abstract.

*Cryptosporidium* is an intracellular protozoan described since 1907 by Tyzzer. Cryptosporidiosis is a cosmopolitan disease, whose incidence is higher in summer and rainy season. The objective of this research was to report the prevalence of *Cryptosporidium* spp. in sheep from 4 commercial sites in 3 Municipalities in the State of Mexico, Mexico in summer. 200 stool samples were taken from pregnant, non-pregnant and nursing ewes and lambs under one year old. All sheep were clinically healthy. A general prevalence of 61% was observed. Texcaltitlán had the highest prevalence (70%). Ewes (63 %) had higher prevalence than lambs (55%). Nursing ewes showed the highest prevalence (67%), which represents a high risk for lambs.

### Introduction

*Cryptosporidium* is an intracellular protozoan described since 1907 by Tyzzer. It classified under the Phylum Apicomplexa, class Sporozoasida, subclass Coccidias, order Eucoccidiorida, and family Cryptosporidiae (Vanathy et al., 2017). The parasites are spherical or elliptical. In the intestinal epithelial cells they can be observed with variable size and are located inside the parasitophorus vacuolae. Oocysts have got four sporozoites, with no sporocysts, which are ovoid and can measure in between 3 to 9  $\mu\text{m}$  according to the species, type and strain (OIE, 2016).

Cryptosporidiosis is a cosmopolitan disease, whose incidence is higher in tropical or warm climate mostly in summer and rainy season. In developing countries, where deficient hygiene and

overpopulation exists, it is posed as a major zoonosis. In epidemiological studies, *Cryptosporidium* has been identified as a common diarrheic cause worldwide (Vanathy *et al.*, 2017).

Neonatal diarrheas in animals cause economic loss, because it delays growth, causes anorexia, weight loss or even animal death. Until the seventies, it was not identified as an agent which could cause diarrheic problems (Abrahamsen *et al.*, 2004). In immunosuppressed animals, it may cause death. It has been identified in young unweaned farm animals such as calves, lambs and kids (Helmy *et al.*, 2013).

Its transmission is fecal-oral, by direct host to host contact or through contaminated food or water that is contaminated with oocysts, and has been identified as the main agent causing epidemics through contaminated water (Vanathy *et al.*, 2017).

The parasite has got a thick wall resistant to conventional chemical treatment, such as 80 ppm chlorine/30 min, and oocysts may remain infective for long periods (Dillingham *et al.*, 2002).

Due to its public health importance, as well as representing one of the main agents involved in neonatal diarrhea, the objective of this research was to report the prevalence of *Cryptosporidium* spp. in sheep from different commercial sites in the State of Mexico, Mexico in summer.

## Materials and Methods

Two hundred stool samples were taken from pregnant, non-pregnant and nursing ewes and lambs under one year old in four commercial sites: Ixtlahuaca (1), San Bernabé Bridge and Cieneguillas de Guadalupe (2), and Texcaltitlan (3) in three different Municipalities from the State of Mexico: Ixtlahuaca (1), Almoloya de Juárez (2) and Texcaltitlán (3). The total number was calculated according to Thrusfield (1990) taking into consideration an infinite sample and a prevalence of 34.3% according to previous studies (Alonso-Fresán *et al.*, 2005).

*Cryptosporidium* spp. was identified in fecal samples using Ziehl-Neelsen stain. Animals were considered as positive when characteristic oocysts (color, shape and size) were identified in the smears (OIE, 2016).

## Results and Discussion

A similar prevalence in the sites was observed, with a general prevalence of 61%. Texcaltitlán had the highest prevalence (70%). Ewes (63 %) had a higher prevalence than lambs (55%). Regarding ewes, nursing sheep showed the highest prevalence (67%). All sheep were clinically healthy. Prevalence results are presented in tables 1, 2 and 3.

**Table 1.- Cryptosporidium spp. PREVALENCE IN SHEEP ACCORDING TO ZOOTECHNICAL FUNCTION IN COMMERCIAL SITES OF THE STATE OF MEXICO**

COMMERCIAL SITE	CATEGORY			Z-N POSITIVE ANIMALS	PREVALENCE
<b>IXTLAHUCA (1)</b> n = 60 samples	EWES	PREGNANT	16	8/16	50%
		NURSING	27	16/27	59%
		NON-PREGNANT	5	4/5	80%
	LAMBS	0-6 MONTHS OLD	12	7/12	58%
<b>PUENTE DE SAN BERNABE (2)</b> n = 80 samples	EWES	PREGNANT	22	11 /22	50%
		NURSING	25	20/25	80%
		NON-PREGNANT	11	8/11	72%
	LAMBS	0-6 MONTHS OLD	22	14/22	63%
<b>CIENEGUILLAS DE GUADALUPE (2)</b> n = 30 samples	EWES	PREGNANT	6	3/6	50%
		NURSING	8	5/8	62%
		NON-PREGNANT	3	1/3	33%
	LAMBS	0-6 MONTHS OLD	13	8/13	61%
<b>TEXCALTITLAN (3)</b> n = 30 samples	EWES	PREGNANT	7	7/7	100%
		NURSING	7	4/7	57%
		NON-PREGNANT	2	1 / 2	50%
	LAMBS	0-6 MONTHS OLD	14	5/14	35%
<b>Total</b>			n=200	126/ 200	63.0%

**Table 2.- GENERAL Cryptosporidium spp. PREVALENCE PER COMMERCIAL SITE**

COMMERCIAL SITE	POSITIVE SAMPLES/ NUMBER OF ANIMAL SAMPLED	PREVALENCE
<b>Ixtlahuaca (1)</b>	35/60	58.3%
<b>Puente de San Bernabé (2)</b>	53/80	66.3%
<b>Cieneguillas de Guadalupe (2)</b>	17/30	56.7%
<b>Texcaltitlan (3)</b>	21/30	70.0%
<b>Total</b>	126/200	63.0%

**Table 3.- Cryptosporidium spp. PREVALENCE PER ZOOTECHNICAL FUNCTION.**

CATEGORY/SUBCATEGORY/ NUMBER OF ANIMALS		PREVALENCE PER SUBCATEGORY	PREVALENCE PER CATEGORY
<b>Ewes 139</b>	Pregnant 50	29/50 = 58%	29/139 = 20.9%
	Nursing 67	45/67 = 67%	45/139 = 32.4%
	Non-pregnant 22	14/22 = 63%	14/139 = 10.1%
	Total		88/139 = 63%
<b>Lambs 61</b>	0 to 6 months of age		34/61=55%

Li *et al.*, (2018) in USA, reported a general prevalence of 17.3%, with 30.6% in lambs and 3.2% in ewes. Alonso-Fresán *et al.* (2016) observed a prevalence of 90-100% in lambs less than 60 days old in Tlalpujahua de Rayón, Michoacán de Ocampo State, Mexico. In this same year, Romero-Salas *et al.*, in the State of Veracruz, Mexico, observed a prevalence of 67.5% in lambs aging less than 2 months. Alonso-Fresán *et al.*, (2005) in the State of Mexico observed a prevalence in sheep of 52.8% in Toluca, 43.4% in Xalatlaco and 30.6% in Jiquipilco Municipalities, with a general prevalence of 32.5%, in which lambs aging less than 6 months showed a prevalence of 32.5% and ewes 35.9%. In contrast, Causapé *et al.*, (2002) in Spain reported a general prevalence in lambs aging less than 3 months of 59%, with 79.4% in diarrheic lambs. Xiao *et al.*, (1993) in USA observed a prevalence of 100% in diarrheic lambs aging 5-10 days, 78.3% in lambs 2-3 weeks old and 17.4% in clinically healthy ewes. When analysing these reports, it can be observed that prevalence is variable not only in different geographical areas, but also regarding age and is higher in lambs. Nevertheless, nursing ewes represent a risk for infecting lambs, since in our study their prevalence was the highest (67%).

## Conclusions

A high prevalence to *Cryptosporidium spp.* was found in all commercial sites. We suggest that due to the high prevalence observed in nursing ewes, they might represent a high risk to the flock, specially for lambs.

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