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RESEARCH ARTICLE

Frequency of *Giardia* spp. and *Cryptosporidium* spp. in Domestic and Captive Wild Animals in the North of Veracruz, Mexico

Alarcón-Zapata, Marco Antonio[1], Romero-Salas, Dora[2*], Chaparro-Gutiérrez, Jenny Jovana[3], González-Hernández, Milagros[4], Ojeda-Chi, Maribel Melina[1] and Serrano-Solís Arturo[1]

[1]Universidad Veracruzana. Facultad de Ciencias Biológicas y Agropecuarias Región Tuxpan. Carretera Tuxpan-Tampico km 7.5, Tuxpan, Veracruz, México. C. P. 92870; [2]Universidad Veracruzana. Facultad de Medicina Veterinaria y Zootecnia, Laboratorio de Parasitología, Unidad de Diagnóstico, Rancho Torreón del Molino. Carretera Federal Veracruz-Xalapa km 14.5, Col. Valente Diaz, Veracruz, México. C. P. 91697; [3]Universidad de Antioquía, UdeA, Facultad de Ciencias Agrarias, Grupo de Investigación CIBAV, carrera 75 #65-87, bloque 46, oficina 225, Medellín, Colombia; [4]Universidad Autónoma de San Luis Potosí. Facultad de Agronomía y Veterinaria. Carretera SLP-Matehuala km 14.5, SLP, México. CP. 78321
*Corresponding author: dromero@uv.mx

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ABSTRACT

Giardia spp. and *Cryptosporidium* spp. are cosmopolitan parasites that infect humans as well as domestic and wild animals. Worldwide, they affect several species of wild animals, mostly mammals. In recent years, reports of wild species have increased, indicating that they play an important role in the spread of these parasites. In Mexico, these parasites have been reported mainly in humans and domestic animals, with no reports in wildlife. In the north of Veracruz, there are conservation areas where various species of native and exotic wildlife cohabit; these sites are closed to cattle farms and used for recreation and tourism, with a potential risk of transmission. Our objective was to determine the frequencies of both parasites in wild animals that inhabit a protected area in northern Veracruz, Mexico. Overall, 17 animal species were surveyed, 12 wild and 5 domestic ones, from which 99 individual fresh feces samples were collected and kept frozen until processing. The oocyst concentration technique and the direct immunofluorescence test with a commercial kit were employed. In all host species analyzed, positive samples were found. In wild animals, the general frequency of *Giardia* spp. was 89.3%, whereas that of *Cryptosporidium* spp. was 94.6%. In domestic animals, the frequencies were 79.0% and 67.4% for *Giardia* spp. and *Cryptosporidium* spp. respectively. This is the first study that reports the frequencies of these protozoans in captive wild and domestic animals in the north of Veracruz, Mexico. We conclude that *Giardia* spp. and *Cryptosporidium* spp. are frequent parasites in wild and domestic animals in the study area; furthermore, four species (*Connochaetes taurinus, Equus burchelli, Anser anser*, and *Trachemys scripta*) have not yet been described as hosts for *Giardia* spp. Further molecular studies are needed to allow the identification of genotypes and their potential transmission among species.

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INTRODUCTION

Giardia spp. and *Cryptosporidium* spp. are opportunistic protozoans of the intestinal tract of a wide range of hosts including humans as well as domestic and wild animals, and both are of medical and veterinary importance (Ryan *et al*., 2021). Infections with these protozoans result in diarrhea, mainly associated with young animals (Santin, 2020). Likewise, bovine adult individuals can be asymptomatic carriers and must therefore be evaluated since they could contribute to reinfection in the herd (Hatam-Nahavandi *et al*., 2019). Within these genera, *Giardia duodenalis* and *Cryptosporidium parvum* causes giardiasis and cryptosporidiosis, respectively, in humans and some mammals. Transmission occurs directly and indirectly by the oral-fecal route, ingesting water or food contaminated with human or animal feces infected with the parasite (Zahedi *et al*., 2020). The onset of clinical signs such as diarrhea, abdominal pain, and weight loss depends on the host's age, immunocompromised status, and number of cysts ingested. Similarly, the infection rate is associated with social and environmental factors, such as poor sanitation, limited access to clean water, and poor living conditions (Hamnes *et al*., 2006).

In wild animals, these parasites have been reported in ungulates, canids, felids, primates, marsupials, and marine mammals, among others. Transmission among species is likely to occur through interactions among humans, domestic animals, and wildlife (Xiao, 2010; Feng and Xiao, 2011; Ryan *et al*., 2021). Therefore, determining the frequency and distribution of these parasites in both domestic and wild animals is important for public health.

Wild animals such as deer and raccoons may play an important role as disseminators of strains/genotypes of *Giardia* spp. with zoonotic potential (Song *et al.,* 2018; Solarczyk *et al.,* 2021). *Cryptosporidium parvum* has been reported in several species of free-ranging wild animals such as the American horse (*Equus ferus caballus*), deer (*Capreolus capreolus, Cervus elaphus*), and wild boar (*Sus scrofa scrofa*) (Wells *et al.,* 2015). In Mexico, there are reports of these parasites in only domestic animals, including cattle, sheep, goat, and dogs, whereas their presence in wild animals has not been documented. For this reason, the objective of the study was to determine the frequencies of *Giardia* spp. and *Cryptosporidium* spp. in wild and domestic animals from the northern zone of Veracruz, Mexico.

MATERIALS AND METHODS

Study area: The study was carried out at the Management Units for the Conservation of Wildlife (UMA) "Isla del Toro", located at the Tamiahua Lagoon in the northern zone of Veracruz (21º 33' 45 N, 97º 30' 45 W) Mexico. Feces from domestic animals from ranches surrounding the Tamiahua Lagoon were collected from February 1 to September 30, 2021.

Sample collection: A convenience sampling was carried out, in which 99 samples of 17 animal species were included, namely zebra (*Equus zebra, n* = 3), water buffalo (*Bubalus bubalis, n* = 8), lechwe (*Kobus leche, n* = 3), fallow deer (*Dama dama, n* = 8), sika deer (*Cervus nippon, n* = 3), red deer (*Cervus elaphus, n* = 5), axis deer (*Axis axis, n* = 8), blackbuck (*Antilope cervicapra, n* = 7), wildebeest (*Connochaetes taurinus, n* = 6), graylag goose (*Anser anser, n* = 2), raccoon (*Procyon lotor, n* = 1), pond slider (*Trachemys scripta, n* = 2), bovine (*Bos taurus n* = 13), porcine (*Sus scrofa domesticus n* = 4), sheep (*Ovis aries n* = 10), horse (*Equus caballus n* = 11), and canine (*Canis lupus familiaris n* = 4). All samples were taken from adult animals. Animals included in this study shared food areas and water sources. Regarding wild species, animal groups cohabit in extensive pens. Adult animals had plastic cattle ID tags and were observed until defecation; immediately afterward, fresh fecal samples were collected from the ground, preventing the feces in direct contact with dirt or debris. In domestic species, fecal samples were collected directly from the animal's rectum with plastic gloves. Each sample was identified, kept refrigerated, and transported to the Parasitology laboratory at the Torreón de Molino Ranch, College of Veterinary Medicine and Zootechnics of Universidad Veracruzana, Veracruz, Mexico. The samples were stored at -20°C until processing.

Immunofluorescence assay: All fecal samples were examined using a commercial immunofluorescence assay (IFA; Merifluor Cryptosporidium/Giardia kit; Meridian Bioscience, Inc.) as described in Geurden *et al.* (2004; 2006). First, 1 g of feces was taken from each sample, suspended in distilled water, and placed in surgical gauze for 1 h to retain debris. Subsequently, it was centrifuged at 3,000 rpm for 5 min, and the pellet was resuspended in distilled water to a volume of 1 mL and centrifuged again. A 20-µL aliquot was obtained and placed on an IFA-stained slide; each sample was examined under a fluorescence microscope at 400x.

Data analysis: The results were captured in an Excel spreadsheet and analyzed with descriptive statistics through the STATA® version 17.0 program, where the frequency and confidence indices (95% CI) were determined.

RESULTS

General results: Of the 99 samples, 56.6% (56/99) were from wild species and 43.4% (43/99) from domestic animals. Of the sampled animals, 84.8% (84/99; CI 75.9–91.0) were positive for *Giardia* spp. and 82.8% (82/99; 73.6–89.4) for *Cryptosporidium* spp.

Wild animal samples: *Cryptosporidium* spp. had a higher frequency, 94.6% (CI 84.2–98.6), than *Giardia* spp., 89.3% (CI 77.4–95.5; Table 1). Except for zebras and blackbuck, which showed frequencies of 66.6% and 71.4%, respectively, the remaining species presented a 100% frequency of *Cryptosporidium* spp. antigens. Similarly, for *Giardia* spp., except for blackbuck, zebras, as well as water buffalo and axis deer, with frequencies of 57.1%, 66.6% and 87.5%, respectively, the remaining species presented a 100% frequency of antigens to this parasite (Table 1).

Domestic animal samples: The general frequency for *Giardia* spp. was 79%. The highest frequencies occurred in canines and bovines, with 100% and 92.3%, respectively (Table 2). The lowest frequency occurred in sheep, with 60%. For *Cryptosporidium* spp. a general frequency of 67.4% was observed, with sheep and dogs presenting the highest frequency of 80.0%; the lowest frequency of 45% was found for horses (Table 2).

DISCUSSION

This study evaluated the presence of *Cryptosporidium* spp. and *Giardia* spp. in wild and domestic animals in Veracruz, Mexico. Both populations are susceptible to these parasites, making them parasites of great zoonotic importance. In the present study, *Giardia* spp. was

Table 1: Frequency of *Giardia* spp. and *Cryptosporidium* spp. in wildlife animals by immunofluorescence test.

Host	"n"	*P	*Giardia* spp. **F (%)	***CI 95%	*P	*Cryptosporidium* spp. **F (%)	***CI 95%
Zebra	3	2	66.6	12.5-98.2	2	66.6	12.5-98.2
Water buffalo	8	7	87.5	46.7-99.3	8	100	59.7-100.0
Lechwe antelope	3	3	100	31.0-100.0	3	100	31.0-100.0
Fallow deer	8	8	100	59.7-100.0	8	100	59.7-100.0
Sika deer	3	3	100	31.0-100.0	3	100	31.0-100.0
Red deer	5	5	100	46.3-100.0	5	100	46.3-100.0
Axis deer	8	7	87.5	46.6-99.3	8	100	59.7-100.0
Blackbuck	7	4	57.1	20.2-88.2	5	71.4	30.2-94.9
Wildebeest	6	6	100	51.6-100.0	6	100	51.6-100.0
Graylag goose	2	2	100	19.8-100.0	2	100	19.8-100.0
Racoon	1	1	100	54.6-100.0	1	100	54.6-100.0
Pond sliper	2	2	100	19.8-100.0	2	100	19.8-100.0
Total	56	50	89.3	77.4-95.5	53	94.6	84.2-98.6

*P: Positive; **F: Frequency; *** CI 95% Confidence Interval.

Table 2: Frequency of *Giardia* spp. and *Cryptosporidium* spp. in domestic animals by immunofluorescence test.

		Giardia spp.			*Cryptosporidium* spp.		
Species	"n"	Positive	Frequency (%)	*CI95%	Positive	Frequency (%)	*CI95%
Bovine	13	12	92.3	62.1-99.6	10	77.0	46.0-93.8
Porcine	4	3	75.0	21.9-98.6	2	50.0	9.2-90.8
Sheep	10	6	60.0	27.3-86.3	8	80.0	44.2-96.4
Horse	11	8	72.7	39.3-92.6	5	45.0	44.2-96.4
Canine	5	5	100.0	46.3-100.0	4	80.0	29.8-98.9
Total	43	34	79.0	63.5-89.4	29	67.4	51.3-80.4

*CI 95% Confidence Interval

identified in 12 different animal species, of which four (*Connochaetes taurinus, Equus burchelli, Anser anser* and *Trachemys scripta*) have not yet been described as hosts for *Giardia* spp., making this study the first report of *Giardia* spp. in wild animals that cohabit in a UMA in Mexico.

The presence of *Giardia* spp. in captive animals and the respective frequencies reported differ widely among studies, ranging from 1 to 89.3% (Matsubayashi *et al.,* 2005; Geurden *et al.,* 2009; Beck *et al.,* 2011; Fajardo-Sánchez *et al.,* 2021).

Although in this study, the presence of *Giardia* spp. in *Anser anser* from Mexico is reported for the first time, there are reports of its presence in different species of wild birds. For example, *G. duodenalis* has been reported in wild geese, swans, and domestic geese, with prevalences of 4% to 29% in Egypt and Poland, respectively (Majewska *et al.,* 2009; Kamel and Abdel-Latef, 2021). This variation in frequencies may indicate an infectious process in the case of high concentrations (Elmberg *et al.,* 2017), whereas at low concentrations, birds might act as mechanical vectors (Majewska *et al.,* 2009; Kamel and Abdel-Latef, 2021).

Likewise, the presence of *Giardia* spp. in zebras (*Equus burchelli* and *E. grevyi*) has previously been evaluated, and until now, the presence of this parasite in these equine species and in testudines has not been reported (Chagas *et al.,* 2019; Karim *et al.,* 2021; Ryan *et al*., 2021). However, in our study, both zebras and turtles were positive for this parasite, indicating that they are susceptible to infection and might play a role in the transmission of these parasites to other animals.

In previous studies, *Giardia duodenalis* infections in water buffalo showed a prevalence of 0.7 to 13% (Abeywardena *et al*., 2014; de Aquino *et al*., 2019), whereas in the present studies, Giardia spp. was observed at a high frequency (87.5%) in buffaloes. These differences could be attributed to the climate, management, test used, and the number of samples analyzed in each study (Feng and Xiao, 2011). Regarding wildebeest, even though it belongs to the Bovidae family, the presence of *Giardia* spp. in this animal species has not been reported, despite recent evaluations (Karim *et al*., 2021). Likewise, in cervids, *Giardia* spp. has been reported through immunofluorescence tests, with low prevalences of 0.15% to 3.8% in roe deer (*Capreolus capreolus*, Beck *et al*., 2011), white-tailed deer (*Odocoileus virginianus*; Trout *et al*., 2003), and Axis deer (Karim *et al*., 2021). In our study, a 100% frequency of *Giardia* spp. was found in several wild ruminants, which differs from previous reports. This can be attributed to the fact that, in this UMA, all mammals share common areas, water sources, and feeders, allowing inter-species transmission by fecal-oral contamination (Trout *et al*., 2003).

Previously, *Cryptosporidium* spp. has been reported in four species of wild ruminants: two antelope species, one gazella species, and one Yak species (Geurden *et al*., 2009). *Cryptosporidium parvum* has been reported in the American horse (*Equus ferus caballus*), roe deer, red deer, Spanish wild deer (Hatam-Nahavandi *et al*., 2019), as well as buffaloes and antelopes (Mugasa *et al*., 2023). It has also been found in wild birds around the world, especially flying birds in habitats close to water resources (Elmberg *et al*., 2017), such as the goose and swan (Wang *et al*., 2021). It is important to mention that the zoonotic species *C. parvum* has been detected in a variety of wild and domestic ruminants and in several species of wild birds (Ryan *et al*., 2021). These reports suggest that both ruminants and wild birds play an important role in transmitting *Cryptosporidium* among animals or from animals to humans.

In Mexico, *Cryptosporidium* has been studied in various species of domestic animals including cattle, sheep, and goats, and the presence of *Giardia* has been investigated in cattle, sheep, and canines; irrespective of the species, the prevalence was higher in young animals (Otero-Negrete *et al.,* 2011; Romero-Salas *et al.,* 2016; Godínez-Galaz *et al.,* 2019). However, this study is the first report of the presence of *Cryptosporidium* spp. and *Giardia* spp. in horses.

Our results concur with several studies in which domestic animals, mainly cattle, buffaloes, sheep, pigs, and horses, were hosts of *Cryptosporidium* spp. and *Giardia* spp., and these animals are also considered potential reservoirs for various species of these parasites (Santin, 2020).

Based on our results, *Giardia* spp. and *Cryptosporidium* spp. circulate at high frequencies in various species of wild and domestic animals that share space and drinking water sources as well as in animals in confinement or intensive production management.

In wild and domestic animals in natural or extensive areas, infections by intestinal protozoans are generally less frequent compared to those in animals in intensive areas or management. In intensive systems, with high animal densities, there are higher concentrations of excrements in the environment, potentially contributing to the transmission of pathogens to humans and wildlife. Some *Cryptosporidium* and *Giardia* species are shared among ungulates, allowing interspecies transmission and keeping these parasites circulating in the environment. Therefore, it could be speculated that the density and confinement of animals, as well as contact with other ungulates and humans, can influence the distribution of *Cryptosporidium* and *Giardia* species in ungulate populations (Zahedi *et al*., 2016; Hatam-Nahavandi *et al*., 2019).

This study was limited by the diagnostic test used since the IFA does neither allow the identification of species nor that of genotypes or subspecies of *Giardia* and *Cryptosporidium* that affect the animals evaluated. In addition, it is of great importance to know the transmission routes.

Conclusions: *Giardia* spp. and *Cryptosporidum* spp. can infect several species of domestic animals as well as wild animals that cohabit in a UMA located on Isla del Toro in the north of Veracruz, Mexico, making them an important source of animal-to-animal and animal-to-human transmission.

Due to the limited information on the transmission route between wild animals and humans or/and other animals, more studies are required to clarify these, along with molecular studies to determine the risks for humans they may have on humans who frequent the site.

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Conflicts of interest: The authors declare that they have no conflicts of interest.

Compliance with ethical standards: Original data derived from the authors' work are presented, which have not been submitted at the same time in different journals.

Statement on the welfare of animals: All procedures performed in studies involving animals were in accordance with and approved by the Bioethics and Animal Welfare Commission of the College of Veterinary Medicine and Zootechnics of Universidad Veracruzana and the Ministry of the Environment and Natural Resources (permit number: DGVS-PIMVS-CR-IN-1067-VER/09) and met the requirements of Mexican law (NOM-059-SEMARNAT-2010).

Author contributions: AZMA and RSD conceived and designed the study. AZMA performed the field work and laboratory analyses. AZMA, RSD, CGJJ, MGH, OCMM, and SSA provided the reagents, materials, and tool analysis. AZMA, RSD, and OCMM were responsible for data curation and statistical analysis. AZMA, RSD, CGJJ, MGH, OCMM, and SSA were involved in the writing, reviewing, and editing of the manuscript.

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