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

Alarcón-Zapata, Marco Antonio¹, Romero-Salas, Dora²*, Chaparro-Gutiérrez, Jenny Jovana³, González-Hernández, Milagros⁴, Ojeda-Chi, Maribel Melina¹ and Serrano-Solís Arturo¹


*Corresponding author: dromero@uv.mx

**ARTICLE HISTORY** (23-174)

Received: May 13, 2023
Revised: July 28, 2023
Accepted: August 7, 2023
Published online: November 25, 2023

**Key words:**
Direct immunofluorescence
Intestinal protozoa
Wildlife
*Giardia*
*Cryptosporidium*.

**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.

**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 (Hammes 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 (Equis 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 (21° 33’ 45” N, 97° 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 (Equis 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 taurus, 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 (Equis 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
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 wildlife 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 may 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; Godinez-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

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Giardia spp.</th>
<th>Cryptosporidium spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>154</td>
<td>100</td>
</tr>
<tr>
<td>Goat</td>
<td>127</td>
<td>100</td>
</tr>
<tr>
<td>Pig</td>
<td>113</td>
<td>100</td>
</tr>
<tr>
<td>Buffalo</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>Water buffalo</td>
<td>89</td>
<td>100</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Giardia spp.</th>
<th><em>n</em></th>
<th>Positive Frequency</th>
<th>Cryptosporidium spp.</th>
<th><em>n</em></th>
<th>Positive Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td></td>
<td>13</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine</td>
<td></td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
<td>10</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horse</td>
<td></td>
<td>11</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canine</td>
<td></td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>43</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

**Acknowledgements:** The authors thank “UMA Isla del Toro” for allowing the study and sample collection as well as MVZ Carlos Peña for support in capturing free-living animals. We also thank the livestock producers who allowed their animals to be sampled and the Parasitology Laboratory staff of the “Torreón del Molino” ranch in the Universidad Veracruzana as well as the undergraduate students for assistance in this study. The authors thank two anonymous reviewers whose comments and suggestions improved this manuscript.

**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-PIMYS-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.

**REFERENCES**


