

## Article

# Molecular Identification of *Cryptosporidium* spp., and *Giardia duodenalis* in Dromedary Camels (*Camelus dromedarius*) from the Algerian Sahara

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**Abstract:** (1) Intestinal microbial parasites are major contributors to the global burden of gastrointestinal disease. Such infections are mainly caused by *Cryptosporidium*, *Giardia duodenalis*, and *Entamoeba histolytica*. These parasites are transmitted either directly or indirectly through oral–fecal routes. Previous reports suggested that camels could play a role in the zoonotic transmission of various clinically and veterinary important intestinal parasites, however, limited data are available on intestinal infections of camels, particularly on a molecular level. We aimed to explore the occurrence of these three parasites in camels (*Camelus dromedarius*) in Algeria. (2) A total of 68 samples—63 stool samples from camels and five from the environment—were collected from two desert regions in Algeria and analyzed using PCR and qPCR methods. (3) Overall, 7% of the camels tested positive for zoonotic subtypes of *Cryptosporidium* spp., while 16% of the camels tested positive for *G. duodenalis*. Two environmental samples also tested positive for *G. duodenalis*. None of the samples were positive for *Entamoeba histolytica*. (4) Our results provide one of the first molecular-based identification of these gut parasites in dromedary camels in Algeria. The presence of *G. duodenalis* in the host and the environment unveils, in part, the circulation route of this parasite. Our results will spearhead further investigations into the prevalence and epidemiology of gut parasites in hoofed animals and raise questions concerning their role in health and disease in the area.

**Keywords:** *Camelus dromedarius*; *Cryptosporidium* spp.; *Giardia duodenalis*; *Entamoeba histolytica*; genotyping; prevalence; Algeria



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## 1. Introduction

Historically, the dromedary camel (*Camelus dromedarius*) is a characteristic animal of economic, social, and cultural importance in the African and Asian desert regions. This animal is also a staple food source for the people of the Sahara [1]. Dromedary camels can be infected by many parasitic species, including protozoa, helminths, and arthropods. These infections cause considerable economic losses due to reduced milk and meat production, as well as stunted growth and mortality cases. Among these parasitic species, *Cryptosporidium* spp., *Giardia duodenalis* (syn. *Giardia intestinalis* or *Giardia lamblia*), and *Entamoeba histolytica* are of zoonotic importance worldwide. These protozoan pathogens infect the digestive tract of a broad range of vertebrates [2,3], causing cryptosporidiosis, giardiasis, and amoebiasis,

respectively, the former two of which are associated with human foodborne and waterborne outbreaks [4,5].

Limited data are available on the epidemiology of these diseases in dromedary camels compared to other livestock. Most studies are solely microscopy-based. Therefore, knowledge on the epidemiology of *Cryptosporidium* spp., *G. duodenalis* and *Entamoeba* spp. from camel-raising areas remains fragmentary. Prevalence rates of *Cryptosporidium* spp. in the dromedary camel range from 0% to 88%. In Africa, the parasite has been reported in Tunisia, Egypt, and Algeria [6–8], and in Iraq and Iran [9,10] in Asia. In this host, *Cryptosporidium parvum*, *C. andersoni*, *Cryptosporidium* rat genotype IV and *Cryptosporidium* camel genotype were detected using molecular approaches [8,11–13]. In Bactrian camel (*Camelus bactrianus*), *C. parvum*, *C. muris*, *C. andersoni*, *C. bovis*, *C. hominis*, *C. ubiquitum*, and *C. occultus* have been found [14–19].

The occurrence of *G. duodenalis* in dromedary, Bactrian, and other camelids has been poorly documented [20–22], with prevalence ranging from 7% to 47% [9,23–25]. To the authors' knowledge, no data are available on genotyping and subtyping of *G. duodenalis* in dromedary camels. The prevalence rates of *Entamoeba* spp. in dromedary camels range from 2% to 20% [9,25]. Among the *Entamoeba* species, *E. histolytica* is the most virulent. Human infections with *E. histolytica* are a global health problem worldwide and are of zoonotic concern. The role livestock plays as potential reservoirs for this species is ambiguous [26].

All three genera display a high degree of genetic diversity and have members that infect livestock. Within *Cryptosporidium*, at least 46 valid species and more than 120 genotypes are recognized [27–29]. Of these, *C. parvum* is the most common zoonotic species infecting animals [30]. *Giardia duodenalis* is considered a complex comprising eight genetically distinct assemblages (genotypes; A-H) [3,29]. Of those, assemblages A and B are the most reported zoonotic genotypes in both animals and humans [3,31], while assemblage E has been frequently found in hoofed animals [31]. Similarly, a high degree of genetic diversity is also noted in *Entamoeba*, which is found in a broad range of hosts [32,33]. To the authors' knowledge, there are no molecular data in the presence of *E. histolytica* and, furthermore, limited molecular data is available on *Cryptosporidium* spp. in dromedary camels.

The present study aimed to investigate occurrence and molecularly characterize *Cryptosporidium* spp., *G. duodenalis*, and *E. histolytica* in dromedary camels (*C. dromedarius*) from two regions located in the Algerian Sahara.

## 2. Results

### 2.1. Occurrence of *Cryptosporidium* Species and Subtypes

*Cryptosporidium* spp. was present in 7% (5/63) of the dromedary camels (Tables 1 and 2). Two infected camels (a male and a female) were aged below three years, two were adults (a male and a female) aged above six years, while data were not available for one. Four samples were identified as *C. parvum* and one as *C. bovis* (Table 1). The *C. parvum* isolates had 99.53% identity with the reference sequence MN914085. The *C. bovis* isolate shared 99.19% identity with the reference sequence EF514234. The *C. parvum* *gp60* sequences were subtyped as IIaA15G2R1, IIaA17G2R1, IIaA18G2R1, and IIdA19G1. The IIaA15G2R1 subtype was detected in two different camels, while the remaining subtypes were detected in three different camels. One camel showed mixed infection with IIaA15G2R1 and IIaA18G2R1. The *C. bovis* sample was not amplified at the *gp60* locus since we would require a different set of primers than the ones utilized in this study. Environmental samples were negative for *Cryptosporidium* spp.

Four *Cryptosporidium*-positive camels from El-Oued region were of Sahraoui breed. These camels were infected with *C. parvum* IIaA15G2R1, IIaA17G2R1, IIaA18G2R1, and *C. bovis*. Additionally, one camel infected with *C. parvum* IIdA19G1 from Tindouf region was of Azaouat breed (Table 2).

**Table 1.** Occurrence of *Cryptosporidium* species/subtypes and *G. duodenalis* in fecal samples from examined dromedary camels.

Region	N° of Examined Fecal Samples	<i>Cryptosporidium</i> spp.			<i>G. duodenalis</i>
		No. of positive samples (camels)	Species identity	Subtypes	No. of positive samples (camels)
El-Oued	37	4	3 × <i>C. parvum</i>	IlaA15G2R1 <sup>a</sup> IlaA17G2R1 IlaA18G2R1 <sup>b</sup>	10 <sup>c</sup>
Tindouf	26	1	1 × <i>C. bovis</i> 1 × <i>C. parvum</i>	- IIdA19G1	0

<sup>a</sup> This subtype was detected in two samples from two different dromedary camels. <sup>b</sup> This subtype was detected in one dromedary camel infected with another subtype IlaA15G2R1 (mixed infection). <sup>c</sup> Genotyping (assemblage determination) was not obtained for *G. duodenalis* positive samples.

**Table 2.** Overall information on the epidemiological data on the overall information as well as the samples infected with *Cryptosporidium* spp. and *Giardia duodenalis*.

Character	Category	No. of Sample	No. of Positive <i>Cryptosporidium</i> spp.	No. of Positive <i>G. duodenalis</i>
Region	Tindouf	26	1	0
	El-Oued	42	4	12
	<b>Total</b>	<b>68</b>	<b>5</b>	<b>12</b>
Source	Stool	63	5	10
	Soil	4	0	1
	Water	1	0	1
	<b>Total</b>	<b>68</b>	<b>5</b>	<b>12</b>
Gender	Male	29	2	5
	Female	27	2	2
	Unknown	7	1	3
	<b>Total</b>	<b>63</b>	<b>5</b>	<b>10</b>
Age	<5 years	26	2	7
	5–10 years	20	2	0
	>10 years	10	0	0
	Unknown	7	1	3
	<b>Total</b>	<b>63</b>	<b>5</b>	<b>10</b>
Breed	Azaouat	10	1	0
	Hamra	2	0	0
	Tergui	2	0	0
	Reguibi	11	0	0
	Mauritanian	1	0	0
	Sahraoui	37	4	10
	<b>Total</b>	<b>63</b>	<b>5</b>	<b>10</b>
Breeding system	Extensive	57	4	1
	Semi-intensive	6	1	9
	<b>Total</b>	<b>63</b>	<b>5</b>	<b>10</b>

## 2.2. Genotyping of *Giardia duodenalis*

*Giardia duodenalis* was detected in 17.6% (12/68) of all the samples, which consisted of 10 stool samples from 63 dromedary camels (Tables 1 and 2). Five positive camels (four males and one female) were aged below three years, two (a male and a female) were aged between four and five years, and for three there were no available data. All ten infected camels were of Sahraoui breed from El-Oued region (Table 2). Among the five examined environmental collections, *G. duodenalis* was detected in one soil and one water sample. The sample obtained from soil was successfully genotyped as an assemblage based on sequence analysis of the *tpi* gene. The sequence shared 100% identity with the reference

sequence EF654686 of *G. duodenalis* assemblage E. The remaining samples could not be genotyped due to the low quantity of the target nucleic acid and/or chimera formation.

### 2.3. Occurrence of *Entamoeba histolytica*

No sample tested positive for *E. histolytica* based on the probe-specific quantitative real-time PCR.

## 3. Discussion

The epidemiology of cryptosporidiosis and giardiasis in dromedary camels from camel-raising areas is poorly known as compared to data available in ruminants. Distinctly variable prevalence rates of *Cryptosporidium* spp. and *G. duodenalis* have been documented in different studies. In Algeria, the prevalence of *Cryptosporidium* spp., ranging from 1.8% to 10%, has been reported, and the study herein is in agreement [8,34–36]. The overall prevalence of *G. duodenalis* in dromedaries was 16%. Infection rates ranging between 7% to 39% have been reported in very few studies from the same hosts in Iraq and Saudi Arabia [9,23,25]. Different factors such as study design, diagnostic methods, and sample size could account for the variation of infection rates across studies.

We identified *C. bovis* and the zoonotic *C. parvum* subtypes IIAA15G2R1, IIAA18G2R1, IIAA17G2R1, and IIDAA19G1. A previous molecular study in Algeria revealed a divergent *C. parvum* subtype (If-like-A22G2), which was genetically related to the *C. hominis* subtype family in two dromedary camels [8]. In addition to *C. parvum* and *C. bovis*, dromedary camels have previously been found to be infected with *C. andersoni*, *Cryptosporidium* rat genotype IV, and *Cryptosporidium* camel genotype in China and Egypt [11,13]. Three identified *C. parvum* subtypes belong to the subtype family IIA, which is mostly found in cattle, while one subtype belongs to the IID subtype family, which is mostly found in small ruminants [37,38]. The *C. parvum* subtypes IIAA15G2R1 and IIAA18G2R1 were detected for the first time in the dromedary camel in Algeria, while IIAA17G2R1 and IIDAA19G1 were detected previously in the same host from Australia and Egypt, respectively [12,13]. The IIAA15G2R1 is a major hyper transmissible zoonotic subtype, is widely distributed, and is responsible for numerous outbreaks of human cryptosporidiosis [37,38]. The remaining identified subtypes have also been detected in humans worldwide, indicating their zoonotic potential [39–43]. Human infections with these subtypes, particularly with IIAA15G2R1, have been reported in different studies from North African and Middle East countries [44]. Consequently, the findings of the present study showed that dromedary camels could play an important role as reservoirs of zoonotic *C. parvum* subtypes. Future investigations should focus on not only camels, but also their handlers and their families.

We identified *G. duodenalis* in dromedary camels, soil, and water. This is the first time that this species was molecularly identified in this host. All ten animals infected were from the same region (El-Oued), while none of the Tindouf hosts were positive. This indicates a possible circulation of *G. duodenalis* amongst the camels residing in the El-Oued region. Notably, however, the animals were not diarrheic at the time of collection. Previously, only assemblages E and A were detected in camelids in China [24,45]. While we were unable to perform genotyping, our results are of significance regarding the transmission dynamics of *G. duodenalis*. This prompted us to investigate the presence of this parasite in the environment. One water and one soil sample were indeed positive for the parasite. This strongly points towards circulation of *G. duodenalis* between hosts and the environment. The finding of *G. duodenalis* in such an extreme environment is of note and should be investigated further.

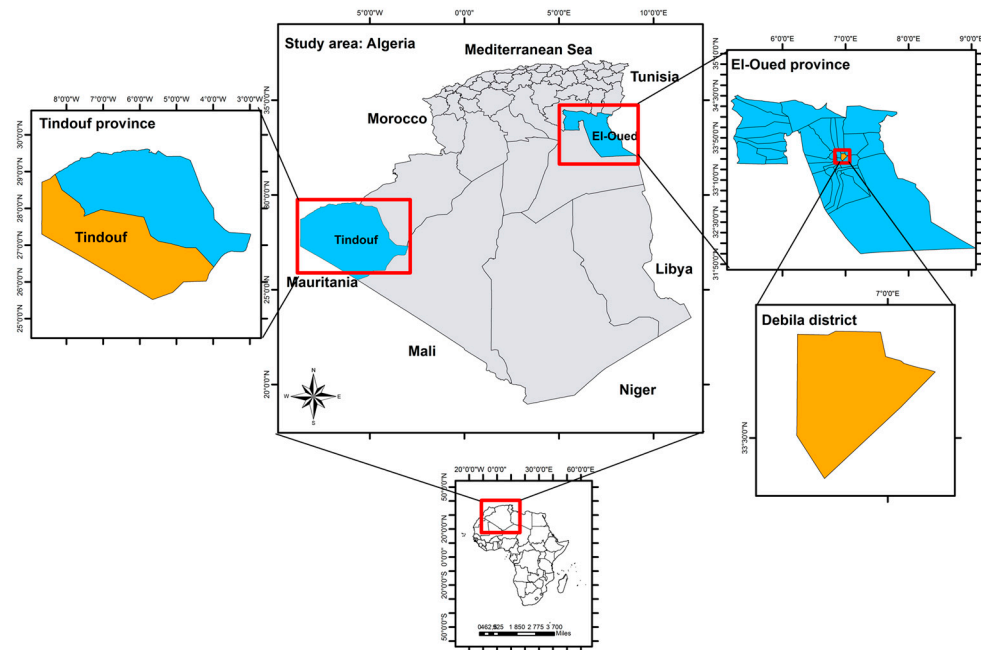
It was worth noting that the occurrence of both parasites was higher in the breed Sahraoui. This predominance was likely attributed to the higher sample size of camels for this breed; all animals examined in El-Oued region were of the Sahraoui breed. Moreover, the difference in the infection rates between the two regions may also be attributed to sample size. Dromedary camels from El-Oued region live in close proximity to sheep and goats. In the Tindouf region, the presence of dogs was observed nearby the camels. This

proximity with small ruminants may be one of reasons for the difference in the infection frequency observed between the two regions, specifically for *G. duodenalis*. A larger study focusing on collecting samples and metadata from all these animals would provide a clearer picture on the role of the hosts/breeds in the transmission dynamics of these parasites.

## 4. Materials and Methods

### 4.1. Study Area and Sample Collection

The present study was conducted between April 2017 and September 2021 in El-Oued and Tindouf provinces of the Algerian Sahara (Figure 1). El-Oued is situated in the north-east of the desert close to the Tunisian border. Tindouf is located in the extreme west of southern Algeria, close to the borders of Morocco and Mauritania.



**Figure 1.** A map illustration of the study sites used for sample collection throughout this study.

A total of 63 fecal samples from seven different farms of dromedary camels (*C. dromedarius*) were collected (Table 2). Twenty-six samples were from four farms in the Tindouf region, while 37 came from three farms in the El-Oued area. Samples were collected immediately after defecation. None of the hosts showed any signs of diarrhea at the time of sampling. Gender, age, breed, and breeding system of the camels were recorded at the time of sampling. Fecal samples were stored in potassium dichromate 2.5% until molecular analyses. Additionally, five environmental samples were collected from El-Oued region. These consisted of four soil samples of 20 g of sand each and they were collected from the field surrounding the camel farms. The one water sample of 40 mL was collected from a tap connected to a ground drinking water source located near the farms.

### 4.2. DNA Extraction, PCRs Analysis, and Sequencing

The genomic DNA (gDNA) from stool and soil samples was extracted using a Pure Link™ Microbiome DNA Purification Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. DNA from the water sample was extracted using E.Z.N.A. Water DNA Kit (OMEGA, Stamford, CT, USA) according to the manufacturer's protocol. The extracted DNA was stored at  $-20^{\circ}\text{C}$  until molecular analysis.

Species determination of *Cryptosporidium* spp. and genotyping were performed using a nested PCR approach to amplify fragments of two genes encoding the small subunit rRNA (SSU, product size:  $\sim 631$  bp) and a 60 kDa glycoprotein (*gp60*, product size;  $\sim 850$  bp) as previously described [30,46–48]. For each SSU-positive sample, three separate *gp60* PCR



amplifications were performed to ensure the detection of mixed infection. All nested PCR products were purified using the Thermo Scientific GeneJET Gel Extraction Kit (Thermo Fisher Scientific, CA, USA) according to the manufacturer's protocol and sequenced bidirectionally using internal PCR primers at Eurofins (Cologne, Germany).

The detection of *G. duodenalis* was performed by probe-based quantitative real-time PCR targeting a fragment of 62 bp of the SSU gene, as previously described [49,50]. Each reaction consisted of a total volume of 20 µL containing 10 µL 1X Luna Universal Probe qPCR Master Mix (New England Biolabs, MA, USA), 0.8 µL of 0.4 µM *G. duodenalis*-specific primers *Giardia*-80F (5'-GACGGCTCAGGACAACGGTT-3') and *Giardia*-127R (5'-TTGCCAGCGGTGTCCG-3'), 0.4 µL of 0.2 µM *G. duodenalis* specific probe (FAM-5'-CCCGCGGCGGTCCCTGCTAG-3'-black hole quencher), 1 µL of 0.5 µg/µL bovine serum Albumin (BSA) (Promega Madison, WI, USA), 5 µL of nuclease-free H<sub>2</sub>O (Promega Madison, WI, USA), and 2 µL of extracted DNA. PCR conditions were as follows: initial denaturation step at 95 °C for 2 min, followed by 50 cycles of 95 °C for 15 s, annealing temperature at 57 °C for 30 s, and a final elongation step at 72 °C for 30 s. The qPCR positive samples were furthermore genotyped through nested amplification of the glutamate dehydrogenase (*gdh*) [51] and triosephosphate isomerase (*tpi*) [52] gene, and through a semi-nested PCR targeting β-giardin (*bg*) [53]. A sample positive with *G. duodenalis* was included as a positive control in each PCR run and a water sample as negative control. Subsequently, positive PCR products were purified using Thermo Scientific GeneJET Gel Extraction Kit (Thermo Fisher Scientific, CA, USA) according to the manufacturer's protocol. Sequencing was performed at Eurofins (Cologne, Germany) in both directions using the internal primers; *tpi*R2 (5'-GTGGCCACCACICCCGTGCC-3') and *tpi*F2 (5'-CCCTTCATCGGIGGTAACCTT-3') if sample(s) tested positive for *tpi*; *gdh*3 (5'-GTGGCGCARGGCATGATGCA-3') and *gdh*4 (5'-ATGACYGAGCTYCAGAGGCACGT-3') if sample(s) tested positive for *gdh*; and *bg*3 (5'-CATAACGACGCCATCGCGGCTCTCAGGAA-3') and *bg*4 (5'-GAGGCCGCCCTGATCTTCGAGACGAC-3') if sample(s) tested positive for *bg*.

*Cryptosporidium* and *G. duodenalis* samples with unclear chromatograms were cloned using the pGEM-T easy vector system I (Promega, Madison, WI, USA) as previously described [54].

The presence of *E. histolytica* was screened by probe-specific quantitative real-time PCR targeting a fragment of 172 bp of the SSU rRNA gene, as previously described [50]. Each reaction consisted of a total volume of 20 µL comprising of 10 µL 1X Luna Universal Probe qPCR Master Mix (New England Biolabs, MA, USA), 0.8 µL of 0.4 µM primers Ehd-239F (5'-ATTGTCGTGGCATCCTAACTCA-3') and Ehd-88R (5'-GCGGACGGCTCATTATAACA-3'), 0.4 µL of 0.2 µM *E. histolytica* specific probe (VIC-5'-TCATTGAATGAATTGGCCATTT-3'-NFQ), 1 µL of 0.5 µg/µL BSA (Promega Madison, WI, USA), 5 µL nuclease-free H<sub>2</sub>O (Promega Madison, WI, USA), and 2 µL of extracted DNA. PCR conditions consisted of an initial denaturation step of 95 °C for 2 min, followed by 50 cycles of 95 °C for 15 s, annealing temperature at 54 °C for 30 s, and final elongation step at 72 °C for 30 s. Positive DNA of an *E. histolytica* isolate was included as a positive control for each PCR run, as well as PCR water as negative control.

#### 4.3. Sequence Analyses

Obtained sequences were visualized and edited manually with the software SnapGeneViewer v.6.0.2, and Chromas pro v.2.1.10. Subsequently, the newly acquired sequences were used as queries to perform BLAST against the GenBank database. Generated nucleotide sequences in the present study were deposited in GenBank under accession numbers OQ613344-OQ613345 for *Cryptosporidium* spp., OQ622075-OQ622079 for *C. parvum* subtypes and OQ622080 for *G. duodenalis*.

## 5. Conclusions

The present study provides molecular data on the occurrence of zoonotic *C. parvum* subtypes and *G. duodenalis* in dromedary camels from two regions of the Algerian Sahara.

Further studies with a larger sample size in the different regions of the Sahara are needed to better understand the epidemiology of cryptosporidiosis, giardiasis, and amoebiasis in these animals, particularly regarding prevalence, host-adapted species/genotypes, and public health significance of the respective responsible pathogens. Sample collections from the herders and their families, along with the surrounding environment, are urgently needed to disentangle the role of camels as sources and/or reservoirs of intestinal parasites.

**Author Contributions:** S.M.; Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing, visualization. N.R. (Nassiba Reghaissiaand); Methodology, resources, writing—review and editing. A.L.; writing—review and editing. H.S.; Methodology. N.R. (Nacira Remdani); methodology. E.G., writing—review and editing, supervision. A.D.T.; Conceptualization, writing—review and editing, supervision. project administration funding acquisition. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data have been submitted to GenBank under accession numbers: OQ613344-OQ613345 for *Cryptosporidium* spp., OQ622075-OQ622079 for *C. parvum* subtypes and OQ622080 for *G. duodenalis*.

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