Prevalence of *Cryptosporidium* spp. in humans and dromedaries (*Camelus dromedarius*) in Algeria

*Nassim Ouchene and Nadjet Amina Khelifi-Touhami**

Abstract

Species of the genus *Cryptosporidium* are among the most important and widespread gastrointestinal parasites in animals and humans. A study was conducted between January and March 2011 to investigate the prevalence of *Cryptosporidium* infection in dromedaries and humans in Oued Souf in southern Algeria. Stool samples of humans (*n*=110) and dromedaries (*n*=40) were collected. *Cryptosporidium* infection was established in human samples by the Heine staining method and by amplification of the 18S rRNA gene using polymerase chain reaction, and in dromedaries by the direct immunofluorescent antibody test. The overall prevalence of *Cryptosporidium* infection in dromedaries was 10% and none of these *Cryptosporidium*-infected animals showed diarrhoea. No significant difference was observed between males (10.52%) and females (9.52%). The number of oocysts calculated per gram of faeces varied between 100 and 450 oocysts/g. In humans, no positive cases were detected using the Heine staining method or 18S rRNA gene amplification. This finding highlighted the presence of *Cryptosporidium* in dromedaries in Oued Souf, Algeria for the first time. Further molecular epidemiology studies including a higher number of dromedaries and different parts of the country are recommended to establish the distribution and national impact of the disease. More efforts are required to isolate and to characterise by PCR the *Cryptosporidium* species in humans in Algeria.

Key words: *Cryptosporidium; dromedary; human, prevalence; Algeria*

Introduction

Cryptosporidium (*Apicomplexa*: *Conoidasida; Cryptogregarinorida*) is a ubiquitous intestinal protozoan parasite that infects the gastrointestinal tract of a wide range of vertebrates including humans, livestock, wild animals and

Nassim OUCHENE, Nadjet Amina KHELIFI-TOUHAMI* (Corresponding author, e-mail: khelifinaa@gmail.com), Institute of Veterinary Sciences, Saad Dahlab University Blida 1, Route Soumâa, BP270, Blida, 09000, Algeria

birds (Caccio, 2005; Fayer et al., 2000; Joachim, 2004; Adl et al., 2019.). There are 44 valid species of *Cryptosporidium* and over 120 genotypes (Ryan et al., 2021). Some species, such as *Cryptosporidium parvum*, are considered zoonotic and a cause of great public health concern (Graczyk et al., 2003; Mammeri et al., 2019).

Cryptosporidium was first discovered in 1907 and identified as a cause of human infection in 1976 (Dubey et al., 1990). In 2004, cryptosporidiosis was added to the World Health Organization's 'Neglected Diseases Initiative', which includes diseases mainly affecting people in low-resource settings (Savioli et al., 2006). The association between cases of cryptosporidiosis and immuno-deficient individuals (AIDS/HIV) brought *Cryptosporidium* to the forefront as a ubiquitous human pathogen (O'Connor et al., 2011).

In immunocompetent persons, *Cryptosporidium*-infection may be asymptomatic or cause a short-term diarrheal illness that resolves spontaneously. In immunocompromised patients, however, *Cryptosporidium* may cause severe, chronic, and possibly life-threatening diarrhoea, and profound malnutrition or wasting (Hunter and Nichols, 2002).

Cryptosporidiosis is more severe in newborn animals and causes severe diarrhoea (Fayer, 2004). However, adult animals are generally asymptomatic carriers and remain a main source of infection to other animals (Xiao et al., 1993).

The prevalence rate of cryptosporidiosis in domestic ruminants (cattle, sheep and goats) worldwide ranges widely, from 0 to 100% (Minas et al., 1994; Quilez et al., 1996; Castro Hermida et al., 2002; Causape et al., 2002; Geurden et al., 2008; Quilez et al., 2008; Ouchene et al., 2012). The distribution and prevalence of *Cryptosporidium* in camels has not been properly investigated and the published information is limited to a few case reports as in the case of Iran (Razawi et al., 2009), China (Wang et al., 2008) and Egypt (Abdel Wahab and Abdel-Maogood, 2011).

In Algeria, data on human and camel cryptosporidiosis are sparsely available. Therefore, the purpose of this study was to investigate the prevalence of *Cryptosporidium* spp. infection in humans and camels in the Oued Souf region of southern Algeria.

Materials and Methods

Sampling procedure

Camel samples

The study was conducted from January to March 2011 in the Oued Souf region of southern Algeria.

Four dromedary farms were selected randomly and faecal samples were collected from ten camels at each farm for a total of 40 samples (19 males and 21 females). The examined camels were crossbred and reared traditionally. For each animal, age and sex were recorded. Faecal samples were collected directly from the rectum using sterile plastic gloves. The samples were transported to the laboratory in a cool box.

Human samples

Stool samples were collected between January and March 2011, at the hospital of the Oued Souf region, southern Algeria. A total of 110 samples were collected. Individual age ranged from 1 to 50 years. Stool samples were classified according to their consistency as diarrhoeic or non-diarrhoeic.

Laboratory analysis

Camel samples

Camel samples (n=40) were concentrated as previously described by Castro-Hermida et al. (2005). Briefly, 2

g faeces from each sample were diluted in distilled water and filtered through a sieve (mesh size: 45 μm) into conical centrifuge tubes. Diethyl ether was then added at a ratio of 2:1, the tubes were shaken vigorously and centrifuged at 1000×g for 5 min at 4°C. The top three layers were decanted off. The sediment was removed and washed in distilled water by centrifugation at 1000×g for 5 min at 4°C. The volume of the oocyst suspension was adjusted to 1 mL using distilled water.

A direct immunofluorescent antibody test (IFAT) (MeriFluor® *Cryptosporidium*/*Giardia*, Meridian Bioscience Europe, Nice, France) was used to search for *Cryptosporidium* spp. oocysts. Briefly, 10 μL oocyst suspension was dried, fixed on the slides using acetone at 4°C for 10 min. The slides were then processed according to the manufacturer's instructions. The oocysts were observed at 400 × magnification under a fluorescence microscope. The number of oocysts per gram of faeces was calculated using the formula: number of oocysts seen in the well × 100/2.

Human samples

Heine staining method

Stool samples (n=110) were analysed with the Heine staining technique for the detection of *Cryptosporidium* spp. oocysts. Demonstration of oocysts was made by staining faecal smears with Carbol-fuchsin and observing at 400 x magnification under a phase-contrast microscope according to Heine (1982).

Oocyst concentration and molecular characterisation

Samples were concentrated as previously described by Castro-Hermida et al. (2005) as described above. Then samples were submitted to DNA extraction; 250 μL oocyst suspension was processed using the UltraClean Faecal DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, USA) according to the manufacturer's instructions.

A two-step nested PCR protocol was used to amplify an 830 bp segment of the 18S rRNA gene using the primers 5′-TTCTAGAGCTAATACATGCG-3′ and 5′-CCCATTTCCTTCGAAACAG-GA-3′ for primary PCR and 5′-GGAAG-GGTTGTATTTATTAGATAAAG-3′ and 5′-AAGGAGTAAGGAACAACCTC-CA-3′ for secondary PCR (Xiao et al., 1999; 2001).

The amplification runs were performed in a Bio-Rad iCycler thermal cycler as following: 10 min at 94°C, 40 cycles of 45 s at 94°C, 55 s at 55°C and 1 min at 72°C, with a final 5 min elongation step at 72°C. Amplification products (10 μL) were analysed on 2% agarose gel and visualised after ethidium bromide staining under UV light.

Furthermore, a drop of each stool sample was observed directly under the light microscope to search for the presence of other gastrointestinal parasites.

Statistical analysis

The chi-square test was used for the statistical analysis. The statistical program used was R i386 3.0.2 for Windows GUI front-end. Differences were considered significant when the P value was less than 0.05.

Results

Camel samples

Four samples from one dromedary farm were positive (two males four and six months of age, and two females five months of age), giving a prevalence of 10%. None of these *Cryptosporidium*-infected animals showed diarrhoea. Prevalence of infection was 10.52% and 9.52% in males and females respectively. No significant difference was observed between males and females. The number of oocysts calculated per gram of faeces varied between 100 and 450 oocysts/g.

Human samples

The Heine staining method and polymerase chain reaction on *Cryptosporidium* spp. revealed no positive samples. On the other hand, other digestive parasites were recorded: *Giardia intestinalis* (47.27%), *Entamoeba coli* (17.27%), *Endolimax nana* (52.72%) and *Pseudolimax butschlii* (5.45%).

Discussion

In this study, we report on the presence of *Cryptosporidium* spp. in dromedaries in the Oued Souf region by using a direct immunofluorescent antibody test. However, no cases were detected in humans by using Heine staining method and molecular analysis by 18S rRNA.

Cryptosporidium infection in camels has been reported in many countries throughout the world (Wang et al., 2008; Ravazi et al., 2009; Nazifi et al., 2010; AbdelWahab and Abdel-Maogood, 2011; Sazmand et al., 2012). In Algeria, from the few studies that have been carried out, only two were conducted by Laatamna et al. (2018) and Baroudi et al. (2018) in the Djelfa and Biskra regions, respectively.

The prevalence of camel cryptosporidiosis reported in different studies worldwide is remarkably variable. The overall prevalence in this study (10%) was in agreement with the prevalence reported in Iran (10%) (Yakhchali and Moradi, 2012), lower than the prevalences report in Egypt (19.3%, 17.5%) (AbdelWahab and Abdel-Maogood, 2011), Iraq (61%) (Hussin et al., 2015) and Iran (37.9%, 20.3% and 16.9%) (Razavi et al., 2009; Nazifi et al., 2010; Sazmand et al., 2012). However, lower prevalences were reported in Algeria (between 2% and 5.1%) (Baroudi et al., 2018; Laatamna et al., 2018), Egypt (3.4%) (Saleh and Mahran, 2007) and Iran (1.9%) (Borji et al., 2009), while no positive cases (0%) were identified in Tunisia, Iraq and Portugal (Mahdi and Ali, 1992; Alves et al., 2005; Soltane et al., 2007).

The variation in the prevalence of *Cryptosporidium* among these different studies could be due to variations in management systems, age, herd and sample size, ecology, study design, season and laboratory techniques employed.

No significant difference was observed between males and females in this study and none of these *Cryptosporidium*-infected animals showed diarrhoea, in corroboration with other reports (Bull et al., 1998; Chalmers et al., 1997; Razavi et al., 2009; Yakhchali and Moradi, 2012; Laatamna et al., 2018). Asymptomatic infected camels may act as healthy carriers of *Cryptosporidium* and may be sources of infection for humans and other animals (Razavi et al., 2009).

In Algeria, little information is available on human cryptosporidiosis. In a study in Oran (northwest Algeria), *Cryptosporidium* was detected only in 4 of 1024 cases giving a prevalence of 0.4% (Benouis et al., 2013). In this study, no positive samples were detected and, in conjunction with previous studies, may indicate a low prevalence of human cryptosporidiosis in Algeria. Indeed, the most important published 2014 report showed that 19 of 27 EU countries reported cases of cryptosporidiosis. Five countries reported zero cases; two reported just one case, and only seven reported 50 or more cases (European Centre for Disease Prevention and Control, 2014). In a study conducted in Melbourne, Australia, 1091 faecal samples from asymptomatic individuals were screened for the presence of *Cryptosporidium* which was detected in only four samples (0.36%) (Hellard et al., 2000). In the Netherlands, microscopy detection screening of 2591 stool samples showed an incidence of *Cryptosporidium* in 0.5 % of cases (ten Hove et al., 2009).

In this study, the prevalence of *Entamoeba coli* and *Pseudolimax butschlii*

was 17.27% and 5.45%, respectively, which is in concordance with the prevalences of 18.95% and 4.43% respectively, in Algeria (Benouis et al., 2013). However, the prevalence of *Giardia intestinalis* (47.27%) and *Endolimax nana* (52.72%) in this investigation were higher than the report in Benouis et al. (2013) (15.32% and 5.24%, respectively).

Conclusion

This finding provides more information on the complexities involved in the host range of *Cryptosporidium* spp. We have proven the presence of *Cryptosporidium* in dromedaries in the Oued Souf region of southern Algeria for the first time. Further molecular epidemiology studies covering a higher number of camels and different parts of the country are recommended to determine the distribution of the species and the national impacts of the disease. The absence of positive cases reported in humans requires more effort to isolate this parasite and to characterise the *Cryptosporidium* species in humans in Algeria.

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Prevalencija roda *Cryptosporidium* u ljudi i jednogrbih deva (*Camelus dromedarius*) u Alžiru

Nassim OUCHENE, Nadjet Amina KHELIFI-TOUHAMI, Institute of Veterinary Sciences, Saad Dahlab University Blida 1, Route Soumâa, BP270, Blida, 09000, Algeria

Kriptosporidiji su jedan od najčešćih gastrointestinalnih parazita u ljudi i velikog broja životinjskih vrsta. Studiju smo provodili od siječnja do ožujka 2011. godine za istraživanje prevalencije infekcije kriptosporidijima u ljudi i jednogrbih deva u mjestu Oued Souf, južni Alžir. Prikupljeni su uzorci ljudskih fecesa (*n*=110) i fecesa jednogrbih deva (*n*=40). Infekcija kriptosporidijima dijagnosticirana je u ljudskim uzorcima pomoću metode bojanja po Heineu te lančanom reakcijom polimerazom, amplifikacijom 18S rRNK gena, a u jednogrbih deva putem izravnog imunofluorescentnog testa za otkrivanje protutijela. Cjelokupna prevalencija infekcije kriptosporidijima u jednogrbih deva bila je 10 %, a niti jedna od životinja zaraženih kriptosporidijima nije pokazivala znakove proljeva; nije zamijećena značajna razlika između mužjaka (10,52 %) i ženki (9,52 %). Broj oocista izračunat po gramu fecesa varirao je od 100 do 450 oocista/g. U ljudi, metoda bojanja po Heineu i 18S rRNK gen nije otkrila pozitivne slučajeve. Ovi nalazi pokazali su, po prvi put u mjestu Oued Souf, Alžir, prisutnost kriptosporidija u jednogrbih deva. Preporučuju se dodatne molekularne epidemiološke studije za utvrđivanje raspodjele vrste i nacionalnog utjecaja bolesti, koje će uključivati veći broj jednogrbih deva i različite dijelove zemlje. Potrebno je i više napora za izoliranje i karakterizaciju vrsta roda *Cryptosporidium* korištenjem PCR, u ljudi u Alžiru.

Ključne riječi: *Cryptosporidium, jednogrba deva, ljudi, prevalencija, Alžir*