Prevalence of cryptosporidiosis in goats in central Algeria

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Abstract

This study was aimed at determining the prevalence of cryptosporidiosis in goats in central Algeria. A total of 605 samples of goat faeces were collected from several mixed farms (sheep and goats) in the regions of Médéa, Djelfa, and Ain Defla. *Cryptosporidium* spp. was detected using the Ziehl-Nelseen technique modified by Henriksen and Polhenz. *Cryptosporidium* spp. was detected in 103 samples (17.02%). Females accounted for 69.90% of positive samples while males accounted for 30.09% of positive samples (P<0.05). The most infested animals were those aged between 2 months and 7 years (69.99%). Animals under 15 days of age were also very vulnerable to infection (59.25%) (P<0.05); Young goats with diarrhoea infested with *Cryptosporidium* were noticed in 25% of samples. The infestation rate during autumn was 56.31%, and 43.68% in spring (P<0.05). Post-weaned goats are potential carriers and shedders of the parasite and young are vulnerable to the infection. To conclude, further studies are needed on a larger sample and in other areas to improve our epidemiology knowledge of cryptosporidiosis in goats.

Key words: *Cryptosporidium; goats; prevalence; age; sex; season*

Introduction

Cryptosporidiosis is an emerging cosmopolitan parasitosis caused by a ubiquitous protozoan of the genus *Cryptosporidium* (Fayer, 2004; Ryan et al., 2014; Starič et al., 2020). Economically, the disease can be the cause of neonatal mortality, weight loss, and the cost of veterinary care (Ceballos et al., 2009; Santín, 2013). In ruminants, the disease manifests mainly with digestive symptoms. Goats, especially young ones under three weeks of age, are most sensitive, often with high morbidity and mortality reaching 100% (De Graaf et al., 1999; Mallinath et al., 2009).

In Algeria, the epidemiological data available on cryptosporidiosis in goats are rare and few studies have examined this topic (Baroudi et al., 2012; Baroudi et al., 2018; Dahmani, 2018; Benhassine,

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2019). Most research has been performed in the north of the country, and nearly all have been qualitative studies testing for *Cryptosporidium* species in young goats to study the agents of neonatal diarrhoea.

The objectives of the present study were to determine the prevalence of cryptosporidiosis in goats of all ages, to correlate diarrhoea and cryptosporidial infection; to determine the most sensitive sex and age, and to determine the impact of season on the onset of the disease in goats in central Algeria.

Material and Methods

Sampling

This study was carried out between March 2017 and March 2019 in mixed farms (sheep and goats); 28 farms in Djelfa, 24 in Médéa, and 6 in Ain Defla. A total of 605 faecal samples were collected. These samples were taken as soon as they were released spontaneously, or after excitation of the anal orifice, and placed into sterile and labelled containers. The consistency of the faeces was recorded at the sampling site. The samples were transported in a cooler to the laboratory (Figure 1).

Detection of *Cryptosporidium* spp.

Microscopic observation of *Cryptosporidium* spp. in faeces depends on the consistency of samples; if the sample is diarrheal, the search can be reduced to the Ritchie concentration technique simplified by Allen and Ridley (Achir and Hamrioui, 2004). On the other hand, if the sample is solid, it becomes imperative that two searching techniques follow one another: Allen and Ridley and stained Ziehl-Neelsen modified by Henriksen and Pohlenz (Henriksen and Pohlenz, 1981).

Richie technique simplified by Allen and Ridley

A few grams of faeces with a volume of 10% formalin water (2 to 3 times greater than that of stool) were placed in a conical stemmed glass tube. The tube WAs shaken until a homogeneous dilution is obtained. After decanting for one to two minutes to remove large faecal debris, part of the supernatant was aspirated and poured into a conical glass tube equivalent to 2/3 of the total volume to be emulsified. A volume of ether corresponding to 1/3 of the total volume to be emulsified was added. After centrifugation at 2500 rpm

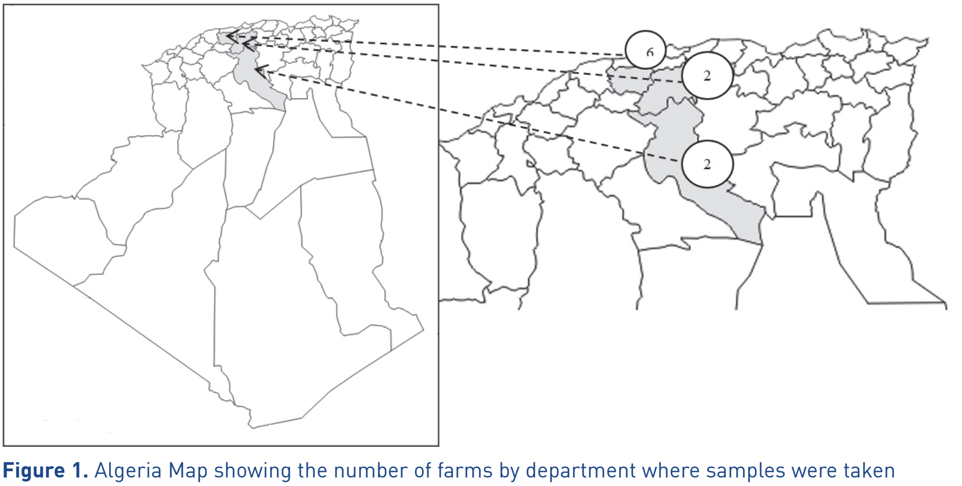

Figure 1. Algeria Map showing the number of farms by department where samples were taken

for 5 minutes, the contents of the tube were distributed into four layers. The three superficial layers are discarded, keeping the pellet in which the parasitic elements are concentrated. A drop of the pellet was placed on a slide and mixed with a drop of Lugol. The slide was covered with a coverslip and examined at a magnification of 10x and then 40x to detect *Cryptosporidium* spp.

Zielhl-Neelsen technique modified by Henriksen and Pohlenz

When stool samples were of a solid consistency, the search for *Cryptosporidium* is continued by the Ziehl-Neelsen method modified by Henriksen and Pohlenz. The faecal smear was made from the centrifugation pellet of the above-mentioned simplified Ritchie method. The smear was fixed in methanol for 5 minutes. Subsequently, it was stained in a carbol fuchsin solution for 60 minutes. After rinsing with water, the smear was counterstained with 5% malachite green solution for 5 minutes, rinsed with water and dried. Reading is done under a microscope at magnifications of 40x and 100x (Figure 2).

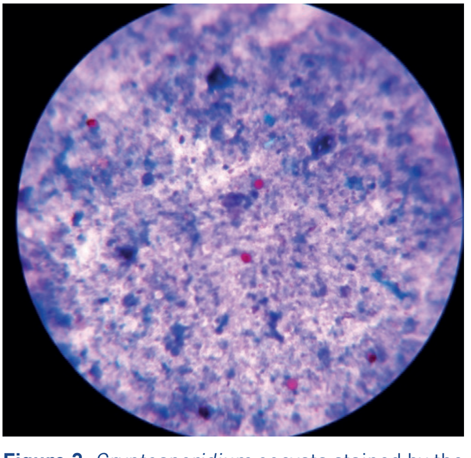

Figure 2. *Cryptosporidium* oocysts stained by the Zielhl-Neelsen technique modified by Henriksen and Pohlenz (magnification 100 x)

Statistical analysis

Descriptive statistics were performed using R software (version 3.5.2). Chi² test of independence was used to examine the links between the rate of Cryptosporidium infection and the various parameters mentioned above and also between the results obtained by the different methods. The confidence interval was calculated for each result. Data were considered significant at P<0.05.

Results

Prevalence of cryptosporidiosis

The results show that all the farms in the study were infested, i.e., 100% (Table 1). The search for the parasite in the faeces of goats revealed that 103 samples out the total 605 (17.02%) were infested by the parasite. The s parasite was not found in 82.97% of the tested samples.

Distribution according to sex, age, and season

The results according to the sex of animals showed that the samples collected from females had a significantly higher rate than those taken from males (69.90% *vs* 30.09%, respectively), P< 0.05.

The data analysis according to the age of the animals revealed that among the samples containing *Cryptosporidium* spp, 16 samples were from animals less than 15 days of age (15.53%); 18 samples from animals between 15 days and 2 months old (17.47%), and the rest of the samples (69) representing the large proportion of goats, aged between 2 months and 7 years (66.90%) (P<0.05).

The treatment of the results in relation to the number of samples from each age group showed that 16/27 (59.25%) samples taken from young less than 15 days old were positive, opposed to 18/87 (20.68%) of those from 15 days to 2 months and 69/491 (14.05%) of those over 2 months and up to 7 years.

Table 1. *Cryptosporidium* spp. depending on sex, age, season and consistency of faeces

Location	N of farms	N of + farms	N of samples	N of + samples	Sex		Age			Season		Consistency	
					male	female	< 15d	[15-2] m	> 2m at 7y	A	S	D	ND
Médéa	25	25 (100%)	203	35 (17.24%)	12 (34.28%)	23 (65.71%)	5 (14.28%)	6 (17.14%)	24 (68.57%)	21 (60.00%)	14 (40%)	4 (12.90%)	31 (88.57%)
Ain defla	7	7 (100%)	83	14 (16.86%)	6 (42.85%)	8 (57.14%)	2 (14.28%)	1 (7.14%)	11 (78.57%)	6 (42.85%)	8 (57.14%)	0 (0%)	14 (100%)
Djelfa	29	29 (100%)	319	54 (16.92%)	13 (24.07%)	41 (75.92%)	9 (16.66%)	11 (25.92%)	34 (57.40%)	31 (57.40%)	23 (42.59%)	10 (18.51%)	44 (81.48%)
Total	61	61 (100%)	605	103 (17.02%)	31 (30.09%)	72 (69.90%)	16 (27)* (15.53%)	18 (87)* (17.47%)	69 (491)* (66.99%)	58 (56.31%)	45 (43.68%)	14 (13.59%)	89 (86.40%)

N: number; *: Number of samples by age group; d: day; m: month; A: Autumn; S: Spring; D: diarrhoea; ND : non-diarrhoea

According to season, data showed that the cases detected during the autumn season were higher than those in spring (56.31% *vs.* 43.68%, respectively), *P*<0.05.

Distribution according to the consistency of faeces

The data in relation to the consistency of the faeces (Table 2) revealed that faeces of normal consistency were clearly higher than that of diarrheal consistency (86.40% *vs* 13.59%, respectively), P<0.05. On the other hand, the treatment of the results according to the age of the animals (Table 2) showed that 4 of the 16 positive samples from young less than 15 days old (25%) were diarrheal and 3 of the 18 positive samples (16.66%) in young aged 15 days to two months had diarrhoea. Taking into account the period of young milk feeding, i.e., less than two months of age, the number of diarrheal samples showed a clear increase, i.e., 7 of the 34 samples (20.58%) against 7 positive diarrheal samples out of 69 (10.14%) samples from weaned kids and goats up to 7 years of age.

Table 2. Consistency of the positive samples

Age	Consistency of faeces	
	D	ND
< 15d	4	16 (25%)
15d-2m	3	18 (16.66%)
2m-7y	7	69 (10.14%)

D: diarrheic; ND: non diarrheic; d: days; m: months; y: years

Discussion and conclusions

In the three, relatively large regions studied, *Cryptosporidium* spp. was detected in samples collected at almost equal rates. These rates explain that *Cryptosporidium* spp. has a homogeneous distribution in these zones and the cryptosporidian infection is maintained and persistent in herds.

To our knowledge, this is the first study of cryptosporidiosis prevalence affecting a significant number of goats in central Algeria. *Cryptosporidium* spp. was found in 103 (17.02%) faecal samples from a total of 605 goats. The parasite was found on all the farms included in the study. This prevalence is significantly lower than that recorded elsewhere in the world. The rate has been estimated at 33.6% in Sri Lanka (Noordeen et al., 2000), 67.9% in Turkey (Duygu, 2017), and 100% in Italy (Drumo et al., 2012). On the other hand, it is similar to the prevalence reported in southeastern Spain (Ceballos et al., 2009), and western France (Castro-Hermida et al., 2005), which were 19.1% and 20%, respectively.

Our results do not agree with the rates advanced in Algeria, which were very low. Benhassine (2019) reported a prevalence of 5.19% for *Cryptosporidium* in a sample of 559 goats. The prevalence found by Baroudi et al. (2018) was slightly higher (8.7%) for goats, mostly in the north of the country on a sample of 92 young goats. Another study conducted in Leon, Spain, reported a rate of 35% in goats (Matos-Fernandez et al., 1993). However, these data are in agreement with the results of Dahmani (2018), who found an incidence of 14.6% in sheep in the region of Ain Oussera in Djelfa. Generally, the prevalence of *Cryptosporidium* spp. ranges from 0 to 100% in goats (Robertson et al., 2014). This means that the parasite could be non-existent or still very widespread.

Our data are especially discussed in relation to local results. The discrepancy between our results and those of Baroudi et al. (2018) could be linked at first to the region of the study but above all to the number of samples examined and the age category targeted by their work, as well as the period of the year when the samples were taken. On the contrary, in the present study, the age of the animals was not chosen as an inclusion or exclusion criterion and sampling was carried out randomly on goats of any age, which may have influenced the data. The difference with other studies around the world can also be explained by several factors; geographical location, and number of animals and farms monitored (Xiao and Herd, 1994; Robertson et al., 2014, Díaz et al., 2018), which differ considerably from one study to another. Regarding the study of Benhassine (2019), it was carried out in the region of Laghouat which represents the Central steppe. The difference there could be associated with the climatic conditions of this zone, characterized by a rather dry and arid climate, as temperatures slightly above 25°C damage the oocysts of *Cryptosporidium* spp. (Olson et al., 2004; Fayer, 2004; King and Monis, 2005).

Regarding the similarity of our data with the study of Dahmani (2018), although it was undertaken on sheep, we believe that it may reflect the health situation of goat cryptosporidiosis for two reasons: firstly due to the cohabitation and simultaneous omnipresence of two animal species that are exposed to the same degrees of infestation by the parasite, and secondly because that study was carried out in an area that was included as part of our study region.

In the present study, the results show a significant difference in the proportion of positive cases in females compared to males, where females were more infected than males. Moreover, these data obtained during the two calving seasons confirm the results reported in several studies. These latter indicated that animals carrying *Cryptosporidium* spp. are mainly females around farrowing, due to the change in the immunoreactivity of domestic ruminants at the end of gestation and during lactation (Xiao and Herd, 1994; Noordeen et al., 2000; Rieux et al., 2013). These females, apparently healthy and carriers of the parasite, are in fact true excretory reservoirs of the disease (Sari et al., 2008).

The ages of the animals were divided into three groups: the first from 0 to 15 days representing the birth of the animal and the period of increased susceptibility of kids to infection (De Graaf et al., 1999); the second from 15 days to two months representing the period of breastfeeding which ends with weaning, and the last over 2 months after weaning when the animal is no longer in close contact with its mother. In this study, the greatest rate of infested animals was found in those over two months of age up to 7 years. The carriage of *Cryptosporidium* spp. by post-weaned animals and adults has been confirmed in small ruminants (Majewska et al., 2000; Ryan et al., 2005; Castro-Hermida et al., 2007; Koinari et al., 2014; Wang et al., 2014; Mi et al., 2014; Kaupke et al., 2017; Diaz et al., 2018). Contamination of older animals can be the result of keeping animals of different ages in confined areas (Khelef et al., 2007; Rahman Raja et al., 2017). These areas are often heavily contaminated by parasites (Akam et al., 2007; Ouchene et al., 2014). These animals, being mostly asymptomatic, eliminate oocysts, which explains the persistence of the disease between ruminants. The shedding of oocysts causes their transmission to newborns and increases the possibility of contamination of several vectors in nature and therefore influences the infection epidemiology (Noordeen et al., 2001).

Although infestation with *Cryptosporidium* spp. has been reported in several studies in farm animals of all ages, its frequency and intensity depend on the age of the animal and has been shown to be more frequent in young animals under one month of age (Thompson et al., 2005; Khelef et al., 2007; Robertson et al., 2014; Bamaiyi and Redhuan, 2016; Naguib et al., 2018). Our results are in agreement with these findings; in fact, the proportion of positive samples found in kids less than 15 days old (59.25%) against 21.83% in those aged 15 days to 2 months and 14.05% in those over 2 months to 7 years old revealed a clear difference in the rate recorded in goats less than 15 days of age.

Regardless of age, sex, place of rearing, more cases were recorded during autumn than in spring. These results are in agreement with those advanced by Noordeen et al. (2001). This slight increase in positive cases during autumn could be explained by climatic variations in the same season (Ceballos et al., 2009).

The data analysis according to the consistency of the faeces shows that the samples of normal consistency were significantly superior to those of diarrheal consistency. The standard error for the calculated confidence interval is large because the sampling locations are geographically distant from each other. The treatment of positive results according to this parameter reveals a strong correlation between diarrhoea and cryptosporidiosis in very young kids (less than 15 days). The data showed that 25% of cases of cryptosporidiosis in kids present a constant symptom, i.e., diarrhoea. This is in agreement with the result of Baroudi et al. (2018).

To conclude, studies previously carried out in Algeria searching for *Cryptosporidium* spp in goats were mostly conducted in the northern regions of the country, where the numbers of small ruminants, in particular goats, are relatively small compared to those in the zones where our study was conducted. In addition, other studies mainly focused on very young animals. Our data show that females were more affected than males around the calving period with no apparent influence of season on infestation by *Cryptosporidium* spp. Young goats (kids) are vulnerable to *Cryptosporidium* infection and a part of these cases presented diarrhoea, which is strongly linked to the infection. Further studies are needed on larger samples and areas to improve our epidemiology knowledge of cryptosporidiosis in goats.

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Prevalencija kriptosporidioze u koza u središnjem Alžiru

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Cilj je ove studije bio ustvrditi prevalenciju kriptosporidioze u koza u središnjem Alžiru. Ukupno je s nekoliko miješanih farmi (ovce i koze) u regijama Médéa, Djelfa i Ain defla prikupljeno 605 uzoraka fecesa koza. Testiranje na *Cryptosporidium* spp. provedeno je putem Ziehl-Nelseen tehnike koju su modificirali Henriksen i Polhenz. *Cryptosporidium* spp. je otkriven u 103 uzoraka (17,02 %). Ženke su bile poztivne 69,90 %, a mužjaci 30,09 % od ukupnih uzoraka (P<0,05). Najzaraženije životinje bile su one u dobi od 2 mjeseca do 7 godina (69,99 %), a vrlo osjetljive na infekciju (59,25 %) (P<0,05) bile su životinje mlađe od 15 dana. U 25 % uzoraka zamijećene su mlade koze s proljevom zaražene kriptosporidijem. Postotak zaraženosti koja je zabilježena tijekom jeseni bila je 56,31 %, u usporedbi s (43,68 %) s proljećem iste godine (P<0,05). Koze odbijene od hrandibe mlijekom potencijalno su prijenosnici parazita i prenose ih, a mlađe životinje su osjetljive na infekciju. Zaključno, potrebne su dodatne studije na većem broju uzoraka i područja da bi se poboljšalo naše epidemiološko znanje o kriptosporidiozi u koza.

Ključne riječi: *Cryptosporidium*, *koze, prevalencija, dob, spol, godišnje doba*