CRYPTOSPORIDIUM SPP. IN SMALL RUMINANT AT THE CENTRAL STEPPE IN ALGERIA: OCCURRENCE AND SANITARY EFFECT

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Abstract. *Cryptosporidium spp*. are diarrhoeic parasites of man and wide ranges of animals with major health implications. To explore their presence in small ruminant from the steppe in Algeria a sampling of 1402 stools was carried out on 875 sheep and 527 goats with different ages, sex and clinic status at many sites from Laghouat, Djelfa and El-Bayadh. Fecal specimens were concentrated and stained by the modified Ziehl-Neelsen acid fast technique. *Cryptosporidium spp*. were identified in 3.77% of sheep and 22.09% of its herds and in 5.12% of goats and 28.36% of hits herds, without age relation neither diarrhea depending, only few cases have been isolated from young diarrhoeic animals. But regarding the type of rearing, it shows strongly correlated to the infection in small ruminant. This study reveals for the first time the occurrence of *Cryptosporidium* in goats and confirms its presence in sheep from steppe in Algeria.

Key words: *Cryptosporidiosis*, Sheep, Goats, Steppe, Occurrence, Diarrhea, Algeria

INTRODUCTION

Cryptosporidium spp. is an enteric protozoan parasite infecting humans and broad ranges of animals that manifests often in diarrheal disease in their hosts (16, 10, 8). Cryptospridiosis in animals can be fatal, it's one of the most incriminate in newborn death in cattle's and goat's herds (7, 28). It costs more when it persists in lambs and goat kids leading to significant economic losses in meat production (7, 27, 2). Additionally infected animals pose great risks to humans since they are implicated in zoonotic transmission (7).

Cryptosporidium spp. is hard to control in animal breeds, it has a very strong resilience in the environment, it can survive for many months in the outside (10, 14). It's widespread in all environments and on most continents (2). In absence of an effective treatment it remains challenging for animal health across the world what increases its threat in animal breeding (21). Many studies have been conducted to investigate this parasite in livestock focusing mostly in cattle unlike goats and sheep that have received little interest (30, 25, 8). More studies on *cryptosporidium* in small ruminant have been done in developed countries but few are that has been released to explore it in developing ones where this two species are more abundant (29, 30). Similarly, in Algeria the most research on animal cryptosporidiosis (1, 13, 19, 3, 4, 18) was driven on cattle. The later forms a small percentage of the Algerian national herd, sheep and goats compose the most of them but the small ruminant cryptosporidiosis relevance remains unclear. In the light of these requirements we have undertaken this study to assess *cryptosporidium* prevalence in sheep and goats in different sites at the central steppe where they are greater in Algeria. And determine the relationship between animal age, type of rearing and the clinic status, with the infection in small ruminant.

MATERIAL AND METHODS

Sites and animal's population study. This study was conducted at the central steppe of Algeria where 60% of sheep and goats are located. In there animal breeding suits best and form the major activity of steppe population. Our sampling was undertaken in the spring kidding season from May 2014 to June 2016 in three sites including the highest number of livestock, Laghouat, Djelfa and El-Bayadh. Altogether, 1402 stools samples were collected at random from 875 sheep and 527goats from both females and males in various age groups with diarrhoeic and no diarrhoeic stools (All diarrhoeic stools have been sampled from young animals). A total of 125 farms were visited with either sheep (n= 58) and goats herds (n= 39) or mixed sheep and goats herds (n= 28) from each one 10 animals at least was submitted to sampling.

Specimen collection. All stools samples were collected directly from the rectum and transferred in plastic containers (2-5g), and then they were transported in an isotherm box to the laboratory and stored at 4°C. Once collected within 1 to 3h, such samples was homogenized with spatula with 2.5% potassium dichromate (v/2v) (Biochem Chemopharma) and stored at 4°C until examination.

Laboratory analysis. On arrival to laboratory and before copromicroscopics analysis, stools were put into tubes and centrifuged for 10 min at 1500 rpm (1500g). After centrifugation, the supernatants corresponding to potassium dichromate was discarded, leaving only the sediments in the tube. Then, faecal sediment was assigned by the Ritchie's concentration technique followed by the modified Ziehl-Neelsen staining for *Cryptosporidium* identification. The microscopic observation was performed in 100 to 150 randomly microscopic fields under ×400 and ×1000 magnifications.

Statistical analysis. The Fisher and Chi-square tests was used in comparisons of infection rates. The analyses were performed using the XLstat 2014 (Microsoft®, WA, USA). Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Cryptosporidium spp. were positive in only 59 samples and 27 among animals and herds examined respectively. Although the highest infection rates were detected in goats 5.12 % compared to sheep 3.77 % from respectively 28.35% and 20.93% herds examined, this rates are lesser than those recorded in many parts of world.

In goats these are the first results that reveal *Cryptosporidium* prevalence in Algeria, it shows lower than that of Spain, where it was reported to be 19.1% (22). *Cryptosporidium* rate can reach average 37%, like what found in Sri Lanka (16), Poland (12) and Ghana (26), and may grow to 67.9% or even 100% according the Turkish and Italian reports (9, 23). Which all presents higher value than the present one. However (24) in Tunisia and (25) in Uganda, report prevalence (0% and 3.5 %) much inferior in this same species.

In sheep, there are more studies on cryptosporidiosis than in goats, prevalence for *Cryptosporidium spp.* commonly ranges from 0 to 77% (30). Little are those with prevalence as low as to ours, in Spain and Brazil, (31, 11, 8) have obtained a law prevalence but this was seen in post-weaned and adult sheep. In Algeria, (6) was the only who has studied ovine cryptosporidiosis, this was in the same area (Ain Oussera), he found prevalence more important than ours either at the individual level (14.6 %) and the flock level (84%).

Some study design factors such as whether only diarrhoeal animals were sampled or not or if is a question of point prevalence study or a longitudinal study account in the variation of prevalence (30, 8), in the present study that contribute to assess the point prevalence, there are a few stools with diarrhea in young animals. This could involve likewise the choice and the performance of the diagnostic tests used. Our samples were performed with microscopic tools that appear less sensitive than immunoassay and PCR methods (30, 15).

Low prevalence of *Cryptosporidium* in the present work is likely related also to the sampling number submitted for microscopic examination from each animal that must be tested more than one time within an interval of a few days which may reveal more false negatives samples (11, 33), in this study the animals were sampled only once. Another explanation is related to climate. Several sites were visited in the steppe with more arid condition which can compromise *Cryptosporidium spp*. survival in this environment (10, 14). In addition temperatures in there are high enough to reduce parasite's spread in the area (14), steppe has a semi-arid climate in which temperature hovering around 20 °C during the year. In such conditions oocysts infectivity is largely affected, it persist only 12 weeks days in the outside (14) which decrease its viability in this environment.

Different reasons can contribute to these reduced rates but not as well as the age factor. It seems that *Cryptosporidium spp.* in these sites are frequent in post-weaned and adult animals which are less susceptible to infection (7, 17), thus less prevalent. In our knowledge the infection has a strong relation with age, what was the same as revealed in this study, but this was seen just in Laghouat for sheep ($P<0.0001$) and El-bayadh for Goats ($P<0.0001$), *Cryptosporidium* was more prevalent in pre-weaned animals in this two sites. Infection in Djelfa and El-Bayadh for sheep and in Laghouat and Djelfa for goats seems to be excluded to the age factor, it was observed in all age groups with similar percentages generally (Table 1) which is unexpected, commonly cryptosporidiosis is age related infection, more rates have been detected in young up to 1 month (11, 30). However (8) in Spain and (26) in Ghana demonstrate also that there is no correlation between age and *Cryptosporidium spp*. in sheep and goats, most positive in these studies was isolated in older animals. Which is the case in Laghouat and El-bayadh for Sheep and Djelfa and Laghouat for goats.

Prevalence can vary depending on differences in which *Cryptosporidium* species is present (30). For the youngest, *C parvum* is one of the most incriminated in cryptosporidiosis for either sheep and goat (32, 23, 2015) which is closely related to the animal age (30), in old animal other genotypes have been implicated in animals in all age groups, may be this was the case of our data. Further investigation is required for better knowledge.

Diarrhea was absent in most positive cases, it appears that *Cryptosporidium* infection is independent to diarrhea. It is therefore asymptomatic. *Cryptosporidium* seems to have an impact on small ruminant only in Laghouat for sheep ($P<0.0001$) and in El-Bayadh ($P=0.001$) for goats, it is more frequent in diarrheoic stools than the non-ones in there. It's therefore also present in no-diarrheoic stools in the other sites (Table 2) in post-weaned and adults, it's in fact asymptomatic in older animals (28, 30). This result suggests the existence of more than one species and/or genotypes with variable virulence. The more pathogenic are in sheep from Laghouat and in Goats from El-Bayadh and the less pathogenic one in the other, which is in accordance with our previous analysis for the correlation between *Cryptosporidium spp.* and animal age. Anyway genotyping here is needed not only to estimate the zoonotic effect of these parasites in this region but to understand clearly how cryptosporidiosis occurs in small ruminant.

Table 1.

Prevalence of *Cryptosporidium spp.* in sheep and goats according to the age groups in the three sites examined at the central Algerian steppe

Age groupes	Sheep				Goats			
	Laghouat	Djelfa	El-Bayadh	Total	Laghouat	Djelfa	El-Bayadh	Total
0-7 D	1/11 (9.09%)	1/19 (5.26%)	0/10 (0%)	2/40 (5%)	0/4 (0%)	0/16 (0%)	0/1 (0%)	0/21 (0%)
8-15 D	1/10 (10%)	02/25 (8%)	0/11 (0%)	3/45 (6.67%)	1/7 (14.28%)	2/19 (10.5%)	4/7 (57.14%)	7/33 (21.2%)
16-21 D	2/16 (12.5%)	0/9 (0%)	1/14 (7.14%)	3/39 (7.69%)	0/3 (0%)	0/6 (0%)	0/3 (0%)	0/12 (0%)
22-30 D	2/19 (10.52%)	3/40 (7.5%)	2/45 (4.44%)	7/104 (6.73%	1/8 (12.5%)	5/44 (11.36%)	0/0 (0%)	6/52 (11.54%)
1-3 M	0/67 (0%)	5/67 (7.46%)	1/113 (0.84%)	6/247 (2.43%)	0/35 (0%)	4/60 (6.67%)	0/31 (0%)	4/126 (3.18%)
4-6 M	0/41 (0%)	43/58 (8.6%)	0/22 (0%)	3/121 (2.48%)	0/33 (0%)	5/37 (13.51%)	0/25 (0%)	5/95 (5.26%)
7-12 M	0/16 (0%)	5/61 (8.19%)	0/4 (0%)	5/81 (6.17%)	0/15 (0%)	3/41 (7.31%)	0/19 (0%)	3/75 (4%)
>1 Y	1/106 (0.94%)	1/39 (2.58%)	2/52 (3.85%)	4/197 (2.03%)	2/78 (2.56%)	0/27 (0%)	0/8 (0%)	2/113 (1.77%)
Total	7/286 (2.45%)	20/318 (6.28%)	6/271 (2.21%)	33/875 (3.77%)	4/183 (2.18%)	19/250 (7.6%)	4/94 (4.25%)	27/527 (5.12%)

D: day; M: month; Y: year

Table 2

Prevalence of *Cryptosporidium spp*. in sheep and goat according to the clinical status in the three sites examined at the central Algerian steppe

Animals	Sheep				Goats			
	Laghouat	Djelfa	El-Bayadh	Total	Laghouat	Djelfa	El-Bayadh	Total
Diarrhoeics	6/18 (33.33%)	2/35 (5.71%)	2/19 (10.53%)	10/72 (13.89%)	0/7 (0%)	0/8 (0%)	2/2 (100%)	2/17 (11.76%)
No Diarrhoeics	1/268 (0.37%)	18/283 (6.36%)	4/252 (1.59%)	23/803 (2.86%)	4/176 (2.27)	19/242 (7.85%)	2/92 (2.17%)	25/510 (4.9%)

On the majority of the farms (92/125), animals were reared under extensive management system, whereas on 33 farms, goats and sheep were reared under the intensive management system in both mixed and non-mixed farms for sheep and goats. Infection of either sheep (38.1% vs 16.92%) (p= 0.042) and goats (55% vs 25.53%) (p=0.012) is high in breeds of intensive management system in comparison with those of extensive management system in all sites (Table 3) which is in agreement with most literature finding (6, 33, 15). The high population densities and animal promiscuity in flock house are in question in this context (30).

In addition, *Cryptosporidium spp.* has been isolated from sheep and goats in many mixed breeds. 35.71% is the rate of infection in mixed breeds against 20.62% in the non-mixed ones, and that confirm the cross-species transmission of this parasite between animals living in the same location (34).

Table 3

Prevalence of positive breeds for *Cryptosporidium spp.* according to the breeding management system in sheep and goats from the three sites sampled in the central Algerian steppe

Breeding management system	Sheep				Goats			
	Laghouat	Djelfa	El-Bayadh	Total	Laghouat	Djelfa	El-Bayadh	Total
Extensive	1/16 (6.25%)	6/25 (24%)	4/24 (16.67%)	11/65 (16.92%)	1/10 (10%)	6/19 (31.57%)	4/18 (22.22%)	12/47 (25.53%)
Intensive	2/11 (18.18 %)	6/10 (60%)	0/0 (0%)	8/21 (38.1%)	3/8 (35.7%)	5/12 (41.67%)	0/0 (0%)	11/20 (55%)
Total	3/27 (11.11)	12/35 (34.28%)	4/24 (16.67%)	19/86 (22.09%)	4/18 (22.22%)	11/31 (35.48%)	4/18 (22.22%)	19/67 (28.36%)

CONCLUSION

From our finding, *Cryptosporidium spp*. shows present in small ruminant breeds at the central steppe in Algeria but with reduced prevalences. It has been isolated the most in animal cohort and seems asymptomatic without age relation with the infection, however the few cases detected in diarrhoeic young animals suggest a health hazard even zoonotic risk in small ruminant in the area.

Hence farther studies must be conducted to explore more sites in the steppe, with some study design considerations given to the number of samples submitted to examination, diagnostic tools (PCR and immunoassay) and with focus on young diarrhoeics animals for better studying cryptosporidiosis of small ruminant in Algeria which is required to understand his effect on animal and man.

REFERENCES

1. Akam, A., Khelef, D., Kaidi, D., Abdulhussain Maria, S, Şuteu, E, Cozma, V. (2002). Epidémiologie de la Cryptosporidiose bovine dans une région de Mitidja de l'Algérie. *Sci. Parasitol*., 2, 22-27.
2. Bamaiyi, P.H., Nur Eliyana M.R., 2016. Prevalence and risk factors for cryptosporidiosis: A global, emerging, neglected zoonosis. *Asian Biomed*., 10, (4), 309-325
3. Baroudi, D., Khelef, D., Hakem, A., Abdelaziz, A., Chen, X., Lysen, C., Roellig, D., Xiao, L. (2017). Molecular characterization of zoonotic pathogens *Cryptosporidium spp*., *Giardia duodenalis* and *Enterocytozoon bieneusi* in calves in Algeria. *Vet. Parasitol*., 8, 66-69.
4. Benhouda, D., Hakem, A., Sannella, A.R., Benhouda, A., Cacciò, S.M. 2017. First molecular investigation of *Cryptosporidium spp.* in young calves in Algeria. *Parasite*, 24, 15.
5. Chartier, C., Mallereau, M.P., Lenfant, D. (1999). Efficacité du lactate d'halofuginone dans la prévention de la cryptosporidiose chez le chevreau nouveau-né. *Rev. Méd. Vét*., 150, 341-348.
6. Dahmani, H., Hakem, A., Oumouna, M. (2017). Epidemiological study of cryptosporidiosis and disease-associated factors in sheep in a steppe region of Algeria (Ain Oussera). *Agriculture - Science and Practice*, 3-4, (103-104), 140-145.
7. De Graaf, D.C., Vanopdenbosch, E., Ortega-Mora, L. M., Abbassi, H., Peeters, J. E. (1999). A review of the importance of cryptosporidiosis in farm animals. *Int. J. Parasitol.*, 29, 1269-1287.
8. Díaz, P., Navarro, E., Prieto, A., Pérez-Creo, A., Viña, M., Díaz-Cao, J.M., López, C.M., Panadero, R., Fernández, G., Díez-Baños, P., Morrondo, P. (2018). *Cryptosporidium* species in

post-weaned and adult sheep and goats from N.W. Spain: Public and animal health significance. *Vet. Parasitol.*, 254,1-5.
9. Drumo, R, Widmer, G., Morrison, L.J., Tait, A., Grelloni, V., D'Avino, N., Pozio, E., Caccio, S.M. (2012). Evidence of host-associated populations of *Cryptosporidium parvum* in Italy. *Appl. Environ. Microbiol.*, 78, (10), 3523–3529.
10. Fayer, R. (2004). *Cryptosporidium*: a water-borne zoonotic parasite. *Vet. Parasitol*., 126, 37-56.
11. Fiuza, V.R.S., Cosendey, R.I.J., Edwards Frazão-Teixeira, E., Santín, M., Fayer, R., de Oliveira, F.C.R. (2011). Molecular characterization of *Cryptosporidium* in Brazilian sheep. *Vet. Parasitol*., 175, (3-4), 360-362.
12. Kaupke, A., Mirosław, M. Michalski, M.M.M., Rzeżutka, A. (2017). Diversity of *Cryptosporidium* species occurring in sheep and goat breeds reared in Poland. *Parasitol. Res*., 116, (3), 871-879.
13. Khelef, D., Saïb, M.Z., Akam, A., Kaidi, R., Chirila, V., Cozma, V., Adjou, K.T. (2007). Épidemiologie de la cryptosporidiose chez les bovins en Algérie. *Rev. Méd.vét.*, 158, (5), 260-264.
14. King, B.J., Monis P.T. (2007). Critical process affecting *Cryptosporidium* oocyst survival in the environment. *Parasitology*, 134, 309-323.
15. Majeed, Q.A.H., El-Azazy, O.M.E., Abdou, N.M.I., Al-Aal, Z.A., El-Kabbany, A.I., Tahrani, L.M.A., AlAzemi, M.S., Wang, Y., Feng, Y., Xiao, L. (2018). Epidemiological observations on cryptosporidiosis and molecular characterization of *Cryptosporidium spp*. in sheep and goats in Kuwait. *Parasitol. Res*., 117, (5),1631-1636.
16. Noordeen, F., Rajapakse, R.P., Faizal, A.C., Horadagoda, N.U., Arulkanthan, A. (2000). Prevalence of *Cryptosporidium* infection in goats selected locations in three agroclimatic zones of Sri Lanka. *Vet. Parasitol*., 93, (2), 95–101
17. Noordeen, F. Horadagoda, N. U. Faizal, A. C M. Rajapakse, R. P V J. Razak, M. A A. Arulkanthan, A. 2002. Infectivity of *Cryptosporidium parvum* isolated from asymptomatic adult goats to mice and goat kids. *Vet. Parasitol*., 103, (3), 217-225
18. Ouakli, N., Belkhiri, A., de Lucio, A., Köster, P.C., Djoudi, M., Dadda, A., Khelef, D., Kaidi, R., Carmena, D., 2018. *Cryptosporidium*-associated diarrhoea in neonatal calves in Algeria. *Vet. Parasitol. Reg. Stud. Reports*, 12, 78-84.
19. Ouchen, N., Ouchen-Khelifi, N.A., Aissi M., Benakhla, A., (2012) Prévalence de *Cryptosporidium spp*. et *Giardia spp*. chez les bovins de la région de Sétif au nord-est de l'Algérie. *Rev. Elev. Med. Vet. Pays Trop.*, 65, (3-4), 53-56.
20. Papanikolopouloua, V., Baroudi, D., Guob, Y., Yuanfei Wangb, Y., Papadopoulosd, E., Lafie, S.Q., Abd El-Tawaba, M.M., Diakoud, A., Giadinisa, N.G., Fengc, Y., Xiao, L. (2018). Genotypes and subtypes of *Cryptosporidium spp*. in diarrheic lambs and goat kids in northern Greece. *Parasitol. int*., 67, (4), 472-475.
21. Paul S., Sharma, D.K., Boral, R., Mishra, A.K., Nayakwadi, S., Banerjee, P.S., Pawaiya, R.S. (2014). Cryptosporidiosis in Goats: a Review. *Adv. Anim. Vet. Sci.*, 2, (3S), 49-54.
22. Sanz Ceballos, L., Illescas, Gomes, P., Sanz Sampelayo, M.R., Gil Extremera, F., Rodriguez Osorio, M. (2009). Prevalence of *Cryptosporidium* infection in goats maintained under semi-extensive feeding conditions in the southeast of Spain. *Parasite*, 16, 315-318.
23. Sayin Ipek, D.N. (2017). Prevalence and molecular characterisation of *Cryptosporidium pp*. in diarrhoeic pre-weaned goat kids reared under traditional farming system in Diyarbakır, Southeastern Anatolia City, Turkey. *Rev. Méd. Vét*., 168, (10-12), 229-234.
24. Soltane, R., Goyot, K., Dei-Cas, E., Ayadi, A. (2007). Prevalence of *Cryptosporidium spp*. (Eucoccidiorida :Cryptosporiidae) in seven species of farm animals in Tunisia. *Parasite*, 14, 335-338.
25. Squire, S., Ryan, U. (2017). *Cryptosporidium* and *Giardia* in Africa: current and future challenges. *Parasit.Vectors*, 10, 195.

26. Squire, S.A., Yanga, R., Robertson, I., Ayic, I., Ryan, U. (2017). Molecular characterization of *Cryptosporidium* and *Giardia* in farmers and their ruminant livestock from the Coastal Savannah zone of Ghana. *Infect. Genet. Evol*., 55, 236-243.
27. Sweeny, J.P.A., Ryan, U.M., Robertson, I.D., Jacobson, C., 2011.*Cryptosporidium* and *Giardia* associated with reduced lamb carcass productivity. *Vet. Parasitol*., 182, (2-4), 127-139.
28. Rieux, A., Paraud, C., Pors, I., Chartier, C. (2013). Molecular characterization of *Cryptosporidium spp.* in pre-weaned kids in a dairy goat farm in western France. *Vet. Parasitol*., 182, (1-3), 268-272.
29. Robertson, L.J., 2009. *Giardia* and *Cryptosporidium* infections in sheep and goats: A review of the potential for transmission to humans via environmental contamination. *Epidemiol. Infect*., 137, 913-921.
30. Robertson, L.J., Bjorkman, C., Axen, C., Fayer, R., 2014. Cryptosporidiosis in farmed animals. In Caccio, S.M., Widmer, G. (Eds.), *Cryptosporidium*: Parasite and disease (pp. 149-236). Austria: Springer-Verlag Wein.
31. Tembue, A.A.M., Alves, L.C.A., Borges, J.C.G., Faustino, M.A.G., Machado, E.L.C. (2006). Ocorrência de *Cryptosporidium spp*. em ovinos no municipio de Ibimirim, estado de Pernambuco. *Ciênc. vet. tróp., Recife-PE*, 9, (1), 41-43.
32. Tzanidakis, N., Sotiraki, S., Claerebout, E., Ehsan, A., Voutzourakis, N., Kostopoulou, D., Stijn, C., Vercruysse, J., Geurden, T., 2014. Occurrence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium spp*. in sheep and goats reared under dairy husbandry systems in Greece. *Parasite*, 21, 45.
33. Utaaker, K.S., Myhr, N., Bajwa, R. S., Joshi, H., Kumar, A., Robertson, L.J. (2017). Goats in the city: Prevalence of *Giardia duodenalis* and *Cryptosporidium spp*. in extensively reared goats in northern India. *Acta. Vet. Scand*., 59, (1), 1-9.
34. Xiao, L., Feng, Y. (2008). Zoonotic cryptosporidiosis. *FEMS Immunol. Med. Microbiol*., 52, 309-323.