Identification of *Cryptosporidium parvum* IIa and IId zoonotic subtype families and *Cryptosporidium bovis* from calves in Algeria

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Keywords:

Cattle, calves, *Cryptosporidium*, zoonoses, Algeria

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Submitted: 15 May 2023
Accepted: 28 August 2023
Online: 15 November 2023
DOI: 10.19182/remvt.37159

Summary

Cryptosporidiosis is a significant disease in calves caused by the parasitic protist *Cryptosporidium*. The infection results in severe symptoms such as diarrhea, dehydration, delayed growth, and weight loss, often leading to mortality and economic losses. This study aimed to detect *Cryptosporidium* spp. in fecal samples from calves in five Algerian provinces. A total of 65 fecal samples from calves were collected from 12 dairy cattle farms in the north-east of Algeria. The presence of the parasites was established by microscopic screening of the oocysts following an immunofluorescence assay (IFA). IFA-positive samples were analyzed by *18S rRNA* PCR-RFLP (restriction fragment length polymorphism) to determine the species. *Cryptosporidium parvum* was subtyped by sequence analysis of the 60 kDa glycoprotein gene. *Cryptosporidium* oocysts were detected microscopically in 41/65 (63%) samples, of which 26/41 (63.4%) were positive by *18S rRNA* PCR-RFLP. Two *Cryptosporidium* species were detected in 24 samples; *C. parvum* (20/24) and *C. bovis* (4/24). *C. parvum* isolates from IIa and IId zoonotic subtype families were detected: IIaA16G2R1 (9/24), IIdA16G1 (4/24), and IIaA15G2R1 (1/24). Thus, calves are reservoirs of zoonotic *C. parvum* subtypes and represent a public health concern.

■ How to cite this article: Sahraoui L., Mammeri M., Thomas M., Chevillot A., Polack B., Vallée I., Follet J., Ain-Baaziz H., Adjou K.T., 2023. Identification of *Cryptosporidium parvum* IIa and IId zoonotic subtype families and *Cryptosporidium bovis* from calves in Algeria. *Rev. Elev. Med. Vet. Pays Trop.*, 76: 37159, doi: 10.19182/remvt.37159

■ INTRODUCTION

Cryptosporidium spp. are common zoonotic gastrointestinal parasites with a broad range of hosts, including humans, livestock, and wildlife (Rahman, Isa & Yusof, 2017). Cattle, especially dairy calves, are widely recognized as common hosts for *Cryptosporidium* spp. with high infection rates and high levels of oocyst shedding (over 1×10^{10}) in feces. These oocysts are adapted to survive in the natural environment, from where they can directly infect other susceptible hosts, including humans. Cattle, and particularly calves, are one of the largest global reservoirs of zoonotic infections. Clinical symptoms of *Cryptosporidium* infection in calves include mild to severe yellowish diarrhea, dehydration, lethargy, poor appetite, weight loss, and growth retardation. The infection can be fatal in a few days, and can cause profound economic disruption to the calf rearing sector (Dinler et al., 2017; Thomson et al., 2017).

To date, 38 different species of *Cryptosporidium* have been identified using molecular diagnostics (Baptista, Cooper & Kissinger, 2021). Previous studies have identified more than ten species of *Cryptosporidium* in dairy cattle, including *Cryptosporidium parvum*, *Cryptosporidium bovis*, *Cryptosporidium andersoni*, and *Cryptosporidium ryanae*, with *C. parvum* being the most common species (Dinler et al., 2017; Thomson et al., 2017).

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Despite these advances, molecular studies on human and animal cryptosporidiosis in Algeria remain rare (Mammeri & Adjou, 2019). Only four studies have carried out molecular characterization of *Cryptosporidium* in calves from Algeria (Ouchene et al., 2016; Baroudi et al., 2017; Benhouda et al., 2017; Ouakli et al., 2018). This study aimed to provide new insights into the occurrence, molecular diversity, and prevalence of *Cryptosporidium* spp. isolates in cattle from five provinces in two different geographical regions of Algeria.

■ MATERIALS AND METHODS

Specimen collection

From November 2015 to March 2017, a total of 65 rectal fecal samples were collected from randomly-selected calves across 12 farms in five provinces in northern Algeria. These provinces are located in the center-north (Algiers, Boumerdès, and Tizi Ouzou) and northeast (Sétif and Souk Ahras) regions (Figure 1).

The farms, designated as F1 to F12 (n=12), were mainly extensive herds (free-ranging) where animals graze outside during the day and are housed in sheds at night (9/12). The remaining three farms were intensive herds, where animals are housed in farm buildings day and night with no access to grazing (Table I).

Most of the sampled calves were under 60 days old, with or without diarrhea, while only three animals were 90, 120, and 180 days old, respectively. The collected fecal samples were stored individually in plastic boxes with potassium dichromate and kept at 4°C until further use, as previously described (Sahraoui et al., 2019).

Sample processing and microscopy screening

All 65 fecal samples were concentrated using 1 g of original fecal matter as per the previously described protocol (Castro-Hermida et al., 2005). The samples were then screened for the presence of *Cryptosporidium* oocysts using direct immunofluorescence assays (IFA) (MeriFluor *Cryptosporidium*/*Giardia*, Meridian Bioscience Europe, Milan, Italy) as per the manufacturer's instructions with modifications as reported previously (Mammeri et al., 2018). The stained slides were examined at 40x magnification under a Leica fluorescent microscope using the Leica Application Suite software (version 4.5.0; Leica Microsystems, Switzerland). The number of oocysts per gram (OPG) was calculated by multiplying the total number of oocysts by the dilution factor.

DNA extraction, 18S rRNA *PCR-RFLP, and* C. parvum gp60 *PCR*

Previous studies have described the DNA extraction and molecular analysis of both the *Cryptosporidium* spp. *18S rRNA* gene by PCR-RFLP (restriction fragment length polymorphism) and the *C. parvum gp60* gene by sequence analysis (Sahraoui et al., 2019). In brief, fecal samples that tested positive for *Cryptosporidium* oocysts by IFA underwent DNA extraction using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. An additional initial step of six freeze-thaw cycles (freezing in liquid nitrogen for 1 minute followed by heating in a 90°C water bath for 1 minute) was performed before DNA extraction. The DNA samples were then stored at -20°C until molecular analysis. Nested PCR was used to amplify an 830 bp fragment of the *18S rRNA* gene to detect *Cryptosporidium* spp. Positive *18S rRNA* products were subjected to PCR-RFLP analysis using two endonucleases, *Ssp*I and *Mbo*II (New England BioLabs, Ipswich, USA), to identify *Cryptosporidium* species. For example, in the case of *C. parvum*, *Ssp*I and *Mbo*II would generate three restriction fragments (449, 267, and 108 bp) and two bands (771 and 769 bp), respectively (Feng et al., 2007). *Cryptosporidium parvum* samples were subtyped by sequencing the 60 kDa glycoprotein gene (*gp60*) in both directions (Genoscreen, Lille, France) as previously described (Gatei et al., 2006). The nucleotide sequences obtained from 14 isolates were deposited in the GenBank database under accession numbers OM305071 to OM305084 (Table I, next pages).

■ RESULTS AND DISCUSSION

In this study, out of the 65 calf fecal samples examined, 41 samples (63%) were found to carry *Cryptosporidium* oocysts after microscopic analysis (Table 1). In other studies, a *Cryptosporidium* prevalence ranging from 13.7 to 84% has been reported in pre-weaned calves (<60 days old) from Algeria (Baroudi et al., 2017; Ouakli et al., 2018). This variation in *Cryptosporidium* infection frequency may be due to the geographical distribution (location of the farms studied), the climatic conditions, the different farming management practices, the season in which samples were collected, the sensitivity and specificity of the detection methods used for screening, the sampling size, the age of the animal involved in the studies, etc. *Cryptosporidium* was detected on 11 of the 12 farms (91%) involved in this study. Our results are consistent with another study performed in ten cattle farms located in the central and eastern regions of Algeria where

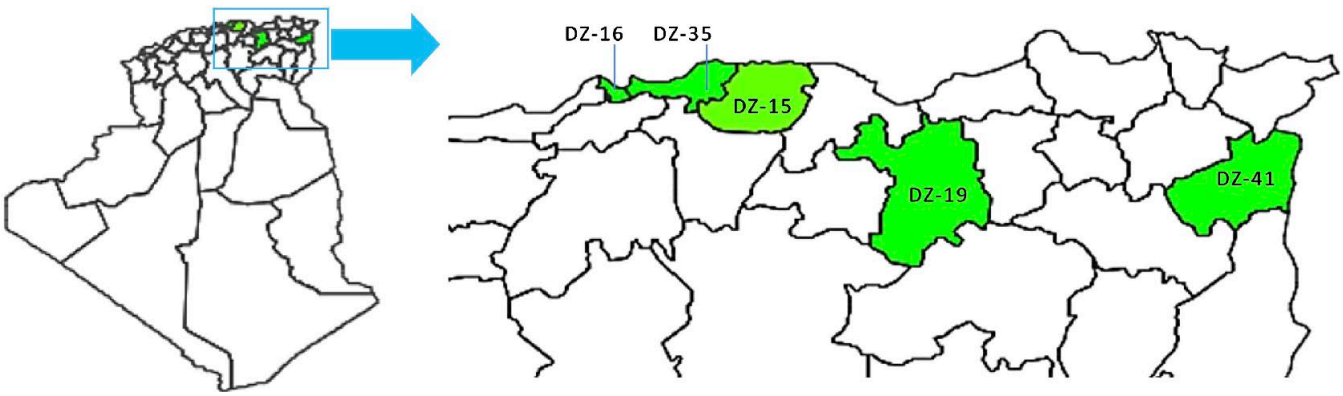

Figure 1. Geographical map of calf fecal sampling locations in Algerian provinces. The number of samples collected from each department (administrative province number-province name) was: DZ-16-Algiers: n=1, DZ-35-Boumerdès: n=1, DZ-15-Tizi Ouzou: n=3, DZ-19-Sétif: n=6, DZ-41-Souk Ahras: n=1 /// *Localisation des lieux d'échantillonnage des fèces de veaux dans les provinces algériennes de l'étude. Le nombre d'échantillons collectés dans chaque département (numéro de la province administrative-nom de la province) est le suivant : DZ-16-Alger : n=1, DZ-35-Boumerdès : n=1, DZ-15-Tizi Ouzou : n=3, DZ-19-Sétif : n=6, DZ-41-Souk Ahras : n=1*

the apparent prevalence at the farm level was 100% (Ouakli et al., 2018). This is in contrast with another study where the prevalence rates ranged from 6% to 100% of positive farms in eastern Algeria (Ouchene et al., 2016).

IFA-positive fecal samples (41/65) revealed that the calves excreted from 10^2 to 1.2×10^6 OPG following oocyst sample concentration. The majority of calves (26/41) were excreting high numbers of oocysts (10^3-10^6 OPG), whereas 15/41 were excreting low levels (< 10^3 OPG). Among the few studies of *Cryptosporidium* excretion levels in cattle in Algeria, rates ranging from 10^2 to 10^4 OPG have been indicated in the east of the country (Ouchene et al., 2016).

Only 26/41 (63.4%) of IFA-positive samples generated PCR amplification products for the *18S RNA* encoding gene. The majority of positive samples originated from calves presenting with diarrhea (21/26) were younger (< 30 days of age), and excreted a high level of oocysts ($6x10^2$ to 1.2×10^6 OPG). The negative samples were generated from animals excreting a relatively low number of oocysts (10^2 to 9.8×10^3 OPG).

Cryptosporidium species were positively identified in 24 of 26 DNA samples by sequence analyses of the *18S rRNA* PCR products. Sequencing results were not able to be interpreted for the remaining two samples (B34 and B39). PCR-RFLP sequence analysis demonstrated the presence of two *Cryptosporidium* species in calves, including *C. parvum* in 20/24 (83.3%) samples from all of the northern Algerian provinces studied, and *C. bovis* in 4/24 (16.6%) specimens from only two provinces. No specific *Cryptosporidium* co-infection pattern was detected. Among the 20 specimens detected as *C. parvum*, only 14 were positive for *gp60* PCR. Three *C. parvum* subtypes were identified with *gp60* gene analysis: IIaA16G2R1 (n=9), IIdA16G1 (n=4), and IIaA15G2R1 (n=1) (Table 1).

Despite positive microscopic identification of oocysts in the samples, the target genes analyzed by *18S rRNA* nested PCR and *gp60* PCR were only positive in 63.4% (26/41) and 70% (14/20) of the samples, respectively. The existence of false negative PCR results could be explained by many factors: low oocyst numbers in some samples (<10^3 OPG), presence of PCR inhibitors in fecal samples (hemoglobin, bilirubin, or bile acids), failed extraction procedures, failed oocyst lysis, insufficient DNA collected, and/or nucleic acid degradation (Johnson et al., 1995; Lantz et al., 1997; McLauchlin et al., 1999). Two species of *Cryptosporidium* were detected in this study, including *C. parvum* (83.3%) and *C. bovis* (16.6%). However, in Algeria and in other countries, in addition to these two species, *C. ryanae* and *C. andersoni* are most often detected in calves (Ouchene et al., 2016; Baroudi et al., 2017; Benhouda et al., 2017; Ouakli et al., 2018; Zhong et al., 2018). Our data shows that calves aged from one to three months were mainly infected with *C. parvum*, while only a few animals were infected with *C. bovis*. This is consistent with the dominant pattern of *C. parvum* infection in young calves (Dinler et al., 2017).

The EU reference center guidelines suggest that genetic characterization of *Cryptosporidium* isolates should be based on two genetic loci and include at least one conserved *18S rRNA* gene (Cacciò et al., 2005). Thus, the *18S rRNA* and *gp60* genes were targeted in our study.

Analysis of the *gp60* gene sequence showed the existence of two families of *C. parvum* subtypes: IIa (10/14) and IId (4/14). Our results are consistent with other studies performed in Algerian calves where the dominant subtype was reported to be IIaA16G2R1 (Baroudi et al., 2017; Ouakli et al., 2018). This subtype is commonly reported worldwide in calves and humans (Feng, Ryan & Xiao, 2018), thus highlighting the zoonotic potential of calves as reservoirs.

To the best of our knowledge, this study reports the presence of the *C. parvum* IIaA15G2R1 and IIdA16G1 subtypes in Algerian calves for the first time. The hyper-transmissible IIaA15G2R1 *C. parvum* subtype has previously been reported to be the most prevalent in calves and humans in many countries. It was reported that within *C. parvum*, the IIa subtype family is panmictic while the IId family is clonal (Feng et al., 2018). In Algeria, animals are rarely traded between regions, which could explain the lower rates of *Cryptosporidium* subtype transmission. The present study is the first to identify the small ruminant IId subtype in Algerian calves. This specific subtype family is prevalent among small ruminants in Algeria (Sahraoui *et al*., 2019). This prevalence potentially poses a contamination risk for calves, particularly on farms where multiple ruminant species are raised together.

Our results also demonstrate that the *Cryptosporidium* population detected in Algeria is more diverse than previous studies would suggest. Consequently, molecular studies in other regions—including in calves and small ruminants—are needed to improve our understanding of cryptosporidiosis epidemiology and *C. parvum* subtype diversity in Algeria.

■ CONCLUSION

Cryptosporidium infection is highly prevalent among calves in Algeria. Molecular studies are necessary for the identification and characterization of the *Cryptosporidium* species/genotypes, and these studies remain rare, especially in Algeria. The zoonotic potential of *Cryptosporidium* in Algeria highlights the need for surveillance and control measures to protect public health.

Conflicts of interest

The author declares that there is no conflict of interest.

Author contributions statement

All authors participated in the conception and design of the study; LS, MM, MT, and AC collected, analyzed, and interpreted data; LS and MM drafted the first version of the article; all authors critically reviewed the manuscript.

REFERENCES

Baptista R.P., Cooper G.W., Kissinger J.C., 2021. Challenges for *Cryptosporidium* Population Studies. *Genes*, **12** (6): 894, doi: 10.3390/genes12060894

Baroudi D., Khelef D., Hakem A., Abdelaziz A., Chen X., Lysen C., Roellig D., et al., 2017. Molecular characterization of zoonotic pathogens *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* in calves in Algeria. *Vet. Parasitol. Reg. Stud. Rep.*, **8**: 66-69, doi: 10.1016/j.vprsr.2017.02.005

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, doi: 10.1051/parasite/2017014

Cacciò S.M., Thompson R.C.A., McLauchlin J., Smith H.V., 2005. Unravelling *Cryptosporidium* and *Giardia* epidemiology. *Trends Parasitol.*, **21** (9): 430-437, doi: 10.1016/j.pt.2005.06.013

Castro-Hermida J.A., Pors I., Poupin B., Ares-Mazás E., Chartier C., 2005. Prevalence of *Giardia duodenalis* and *Cryptosporidium parvum* in goat kids in western France. *Small Rumin. Res.*, **56** (1): 259-264, doi: 10.1016/j.smallrumres.2004.06.007

Dinler C., Ulutas B., Dinler C., Ulutas B., 2017. Cryptosporidiosis in ruminants: Update and current therapeutic approaches. *Am. J. Anim. Vet. Sci.*, **12** (3): 96-103, doi: 10.3844/ajavsp.2017.96.103

Feng Y., Ortega Y., He G., Das P., Xu M., Zhang X., Fayer R., et al., 2007. Wide geographic distribution of *Cryptosporidium bovis* and the deer-like genotype in bovines. *Vet. Parasitol.*, **144** (1-2): 1-9, doi: 10.1016/j.vetpar.2006.10.001

Feng Y., Ryan U.M., Xiao L., 2018. Genetic Diversity and Population Structure of *Cryptosporidium*. *Trends Parasitol.*, **34** (11): 997-1011, doi: 10.1016/j.pt.2018.07.009

Gatei W., Hart C.A., Gilman R.H., Das P., Cama V., Xiao L., 2006. Development of a multilocus sequence typing tool for *Cryptosporidium hominis*. *J. Eukaryot. Microbiol.*, **53** (1): S43-48, doi: 10.1111/j.1550-7408.2006.00169.x

Johnson D.W., Pieniazek N.J., Griffin D.W., Misener L., Rose J.B., 1995. Development of a PCR protocol for sensitive detection of *Cryptosporidium* oocysts in water samples. *Appl. Environ. Microbiol.*, **61** (11): 3849-3855, doi: 10.1128/aem.61.11.3849-3855.1995

Lantz P.G., Matsson M., Wadström T., Rådström P., 1997. Removal of PCR inhibitors from human faecal samples through the use of an aqueous two-phase system for sample preparation prior to PCR. *J. Microbiol. Methods*, **28** (3): 159-167, doi: 10.1016/S0167-7012(97)00979-2

Mammeri M., Adjou K.T., 2019. Veterinary and public health importance of cryptosporidiosis in Algeria: an update and new insights. *Rev. Med. Vet.*, **170** (7/9): 164-173

Mammeri M., Chevillot A., Thomas M., Polack B., Julien C., Marden J.P., Auclair E., et al., 2018. Efficacy of chitosan, a natural polysaccharide, against *Cryptosporidium parvum in vitro* and *in vivo* in neonatal mice. *Exp. Parasitol.*, **194**: 1-8, doi: 10.1016/j.exppara.2018.09.003

McLauchlin J., Pedraza-Díaz S., Amar-Hoetzeneder C., Nichols G.L., 1999. Genetic characterization of *Cryptosporidium* strains from 218 patients with diarrhea diagnosed as having sporadic cryptosporidiosis. *J. Clin. Microbiol.*, **37** (10): 3153-3158, doi: 10.1128/JCM.37.10.3153-3158.1999

Ouakli N., Belkhiri A., de Lucio A., Köster P.C., Djoudi M., Dadda A., Khelef D., 2018. *Cryptosporidium*-associated diarrhoea in neonatal calves in Algeria. *Vet. Parasitol. Reg. Stud. Rep.*, **12**: 78-84, doi: 10.1016/j.vprsr.2018.02.005

Ouchene N., Ouchene-Khelifi N.A., Khelifi M., Zeroual F., Bitam I., Benakhla A., Kaidi R., et al., 2016. Prevalence and Molecular Characterization of *Cryptosporidium* in Dairy Cattle from Farms in Algeria. *Kafkas Univ. Vet. Fak. Derg.*, doi: 10.9775/kvfd.2016.15192

Rahman R.N.R.I.R.A., Isa M.L.M., Yusof A.M., 2017. A review of *Cryptosporidium* spp. infection in livestock. *J. Teknol.*, **79** (6), doi: 10.11113/jt.v79.10330

Sahraoui L., Thomas M., Chevillot A., Mammeri M., Polack B., Vallée I., Follet J., et al., 2019. Molecular characterization of zoonotic *Cryptosporidium* spp. and *Giardia duodenalis* pathogens in Algerian sheep. *Vet. Parasitol. Reg. Stud. Rep.*, **16**: 100280, doi: 10.1016/j.vprsr.2019.100280

Thomson S., Hamilton C.A., Hope J.C., Katzer F., Mabbott N.A., Morrison L.J., Innes E.A., 2017. Bovine cryptosporidiosis: impact, host-parasite interaction and control strategies. *Vet. Res.*, **48**, doi: 10.1186/s13567-017-0447-0

Zhong Z., Dan J., Yan G., Tu R., Tian Y., Cao S., Shen L., et al., 2018. Occurrence and genotyping of *Giardia duodenalis* and *Cryptosporidium* in pre-weaned dairy calves in central Sichuan province, China. *Parasite*, **25**: 45, doi: 10.1051/parasite/2018046

Table I: Molecular characterization of *Cryptosporidium* from clinically-affected pre-weaned Algerian calves, including age and farm location data. /// *Caractérisation moléculaire de* Cryptosporidium *provenant de veaux algériens pré-sevrés cliniquement affectés, incluant les données relatives à l'âge et à la localisation de la ferme.*

Samples (n=65)	Province	Number of the farms	Farms designation	Intensive (I) or Extensive (E) breeding system	Age (days)	OPG	PCR (*18S rRNA*)	PCR (*18S rRNA*) + RFLP	Sequencing (*gp60*)	Access number (*gp60*)
B1	19	6	F1	E	45	8,42E+05	Positive	*C. parvum*	IIaA16G2R1	OM305071
B2			F2	I	38	4,00E+02	Negative	ND	ND	/
B3					30	0,00E+00	ND	ND	ND	/
B4					39	0,00E+00	ND	ND	ND	/
B5					40	0,00E+00	ND	ND	ND	/
B6					20	1,30E+04	Positive	*C. parvum*	IIaA16G2R1	OM305072
B7			F3	I	30	0,00E+00	ND	ND	ND	/
B8					30	0,00E+00	ND	ND	ND	/
B9					30	5,00E+02	Negative	ND	ND	/
B10					32	0,00E+00	ND	ND	ND	/
B11					26	1,20E+06	Positive	*C. parvum*	IIdA16G1	OM305073
B12			F4	E	41	0,00E+00	ND	ND	ND	/
B13					30	7,80E+05	Positive	*C. parvum*	IIaA16G2R1	OM305074
B14			F5	E	45	0,00E+00	ND	ND	ND	/
B15			F6	E	17	1,00E+03	Positive	*C. parvum*	NU	/
B16					15	1,00E+04	Positive	*C. parvum*	IIaA16G2R1	OM305075
B17					21	9,33E+05	Positive	*C. parvum*	IIaA16G2R1	OM305076
B18					120	0,00E+00	ND	ND	ND	/
B19					17	9,00E+03	Negative	ND	ND	/
B20					6	0,00E+00	ND	ND	ND	/

n = total number of samples; *18S = 18S rRNA*; *gp60* = 60 kDa glycoprotein; NA = No Amplification; NU = Non-Usable; ND = Not Done. Department number: DZ-16-Algiers: n=1, DZ-35-Boumerdès: n=1, DZ-15-Tizi Ouzou: n=3, DZ-19-Sétif: n=6, DZ-41-Souk Ahras: n=1 /// *n = nombre total d'échantillons ; 18S = ARNr 18S ; gp60 = glycoprotéine 60 kDa ; NA = pas d'amplification ; NU = non utilisable ; ND = non fait. Numéro de département : DZ-16-Alger : n=1, DZ-35-Boumerdès : n=1, DZ-15-Tizi Ouzou : n=3, DZ-19-Sétif : n=6, DZ-41-Souk Ahras : n=1.*

Table I (continued): Molecular characterization of *Cryptosporidium* from clinically-affected pre-weaned Algerian calves, including age and farm location data. /// *Caractérisation moléculaire de* Cryptosporidium *provenant de veaux algériens pré-sevrés cliniquement affectés, incluant les données relatives à l'âge et à la localisation de la ferme.*

Samples (n=65)	Province	Number of the farms	Farms designation	Intensive (I) or Extensive (E) breeding system	Age (days)	OPG	PCR (*18S rRNA*)	PCR (*18S rRNA*) + RFLP	Sequencing (*gp60*)	Access number (*gp60*)
B21	41	1	F7	E	4	1,00E+02	Negative	ND	ND	/
B22					12	3,69E+04	Positive	*C. parvum*	IIaA15G2R1	OM305077
B23					15	1,00E+02	Negative	ND	ND	/
B24					20	2,70E+03	Positive	*C. parvum*	NU	/
B25					3	2,00E+02	Negative	ND	ND	/
B26					6	3,61E+04	Positive	*C. parvum*	IIdA16G1	OM305078
B27					90	1,43E+04	Positive	*C. parvum*	IIaA16G2R1	OM305079
B28					4	0,00E+00	ND	ND	ND	/
B29					10	9,20E+05	Positive	*C. parvum*	IIdA16G1	OM305080
B30					7	3,00E+02	Negative	ND	ND	/
B31					5	3,00E+02	Negative	ND	ND	/
B32	16	1	F8	E	8	3,20E+04	Positive	*C. bovis*	ND	/
B33					15	7,00E+03	Positive	*C. parvum*	NU	/
B34					26	6,00E+02	Positive	NU	NU	/
B35					3	0,00E+00	ND	ND	ND	/
B36					11	4,50E+02	Negative	ND	ND	/
B37					180	5,00E+02	Negative	ND	ND	/
B38					17	1,60E+04	Positive	*C. parvum*	NU	/
B39					4	8,00E+02	Positive	NU	ND	/
B40					7	0,00E+00	ND	ND	ND	/
B41	15	3	F9	E	45	0,00E+00	ND	ND	ND	/
B42					30	4,60E+03	Positive	*C. parvum*	IIdA16G1	OM305081
B43					40	3,00E+02	Negative	ND	ND	/
B44					45	0,00E+00	ND	ND	ND	/
B45			F10	E	38	1,40E+03	Positive	*C. parvum*	IIaA16G2R1	OM305082
B46					7	0,00E+00	ND	ND	ND	/
B47					7	9,80E+03	Negative	ND	ND	/
B48					50	0,00E+00	ND	ND	ND	/
B49					40	0,00E+00	ND	ND	ND	/
B50					15	4,00E+02	Negative	ND	ND	/
B51			F11	I	21	1,05E+03	Positive	*C. bovis*	ND	/
B52					30	1,80E+03	Positive	*C. bovis*	ND	/
B53					60	0,00E+00	ND	ND	ND	/
B54					60	0,00E+00	ND	ND	ND	/
B55					45	5,50E+02	Negative	ND	ND	/
B56					21	3,00E+03	Positive	*C. bovis*	ND	/
B57	35	1	F12	E	21	1,45E+03	Positive	*C. parvum*	IIaA16G2R1	OM305083
B58					30	1,65E+03	Positive	*C. parvum*	IIaA16G2R1	OM305084
B59					40	4,00E+02	Negative	ND	ND	/
B60					45	0,00E+00	ND	ND	ND	/
B61					40	4,00E+03	Positive	*C. parvum*	NU	/
B62					40	0,00E+00	ND	ND	ND	/
B63					40	4,00E+03	Positive	*C. parvum*	NU	/
B64					40	0,00E+00	ND	ND	ND	/
B65					48	0,00E+00	ND	ND	ND	/
Total	**5**	**12**	**F12**	**9 E ; 3 I**	**/**	**41/65**	**26/41**	**24/26 *C. parvum*: 20/24 *C.bovis:* 4/24**	**14/20 IIaA16G2R1: 9/14 IIdA16G1: 4/14 IIaA15G2R1: 1/14**	**/**

Résumé

Sahraoui L., Mammeri M., Thomas M., Chevillot A., Polack B., Vallée I., Follet J., Ain-Baaziz H., Adjou K.T. Identification des familles de sous-types zoonotiques *Cryptosporidium parvum* IIa et IId et de *Cryptosporidium bovis* chez les veaux en Algérie

La cryptosporidiose est une maladie importante chez les veaux, causée par le protiste parasite *Cryptosporidium*. L'infection entraîne des symptômes graves tels que la diarrhée, la déshydratation, un retard de croissance et une perte de poids, entraînant souvent la mortalité et des pertes économiques. Cette étude visait à détecter *Cryptosporidium* spp. dans des échantillons fécaux de veaux dans cinq provinces algériennes. Au total, 65 échantillons fécaux de veaux ont été collectés dans 12 élevages de bovins laitiers du nord-est de l'Algérie. La présence des parasites a été établie par criblage microscopique des oocystes suite à un test d'immunofluorescence (IFA). Les échantillons positifs à l'IFA ont été analysés par une PCR-RFLP (Restriction Fragment Length Polymorphism) ciblant le gène *18S rRNA* pour déterminer l'espèce. *Cryptosporidium parvum* a été sous-typé par analyse de séquence du gène de la glycoprotéine de 60 kDa. Les oocystes de *Cryptosporidium* ont été détectés au microscope dans 41/65 (63%) échantillons, dont 26/41 (63,4%) étaient positifs en PCR-RFLP *18S rRNA*. Deux espèces ont été déterminées pour 24 échantillons comme *C. parvum* (20/24) et *C. bovis* (4/24). Des isolats de *C. parvum* appartenant aux deux familles de sous-types zoonotiques, IIa et IId, ont été détectés : IIaA16G2R1 (9/24), IIdA16G1 (4/24) et IIaA15G2R1 (1/24). Les veaux sont des réservoirs de sous-types zoonotiques de *C. parvum* et représentent un problème de santé publique.

Mots-clés : Bovin, veau, *Cryptosporidium*, zoonose, Algérie.

Resumen

Sahraoui L., Mammeri M., Thomas M., Chevillot A., Polack B., Vallée I., Follet J., Ain-Baaziz H., Adjou K.T. Identificación de las familias de subtipos zoonóticos IIa y IId de *Cryptosporidium parvum* y de *Cryptosporidium bovis* en terneros de Argelia

La criptosporidiosis es una enfermedad importante en los terneros, causada por el protista parásito *Cryptosporidium*. La infección provoca síntomas graves como diarrea, deshidratación, retraso en el crecimiento y pérdida de peso, comportando a menudo mortalidad y pérdidas económicas. El objetivo de este estudio era detectar *Cryptosporidium* spp. en muestras fecales de terneros en cinco provincias argelinas. Se recogieron un total de 65 muestras fecales de terneros en 12 explotaciones bovinas lecheras del nordeste de Argelia. La presencia de los parásitos se estableció mediante cribado microscópico de oocistos en respuesta a una prueba de inmunofluorescencia (IFA). Las muestras positivas al IFA se analizaron mediante un PCR-RFLP (*Restriction Fragment Length Polymorphism*) enfocado al gen *18S rRNA* para determinar la especie. *Cryptosporidium parvum* fue subcaracterizado por análisis de secuencia del gen de la glicoproteína de 60 kDa. Los oocistos de *Cryptosporidium* se detectaron mediante microscopio en 41/65 (63 %) muestras, de las cuales 26/41 (63,4%) fueron positivas en el PCR-RFLP *18S rRNA*. Se determinaron dos especies para 24 muestras: *C. parvum* (20/24) y *C. bovis* (4/24). Se detectaron cepas aisladas de *C. parvum* pertenecientes a dos familias de subtipos zoonóticos, IIa y IId: IIaA16G2R1 (9/24), IIdA16G1 (4/24) y IIaA15G2R1 (1/24). Los terneros son reservas de subtipos zoonóticos de *C. parvum* y representan un problema de salud pública.

Palabras clave: Ganado bovino, ternero, *Cryptosporidium*, zoonosis, Argelia