

Prevalence of *Cryptosporidium* sp in dairy calves in north-eastern Algeria

N. OUCHENE¹, A. BENAKHLA¹, N.-A. KHELIFI¹, S. RIGHI¹, C. PARAUD², C. CHARTIER^{3*}

¹Centre Universitaire d'El Tarf, Institut des Sciences Vétérinaires, 36000, BP73, El Tarf, ALGÉRIE.

²Anses Laboratoire de Niort, 60 rue de Pied de Fond, B.P. 3081, Niort, F-79012, FRANCE.

³LUNAM Université, ONIRIS, Ecole Nationale Vétérinaire, Agroalimentaire et de l'Alimentation, Nantes, F-44307, FRANCE.

*Corresponding author: christophe.chartier@oniris-nantes.fr

SUMMARY

Parasitological investigations were carried out in dairy calves in Algeria to determine the prevalence and the intensity of oocyst output of *Cryptosporidium* sp infection according to the age and the relationship with the diarrhoea. Fresh faecal samples were collected from 566 less than 12 month-old dairy calves from 40 cattle herds located in 4 regions of north-eastern Algeria. Faeces were processed through concentration and modified Ziehl-Neelsen staining techniques and the presence of *Cryptosporidium* sp oocysts was scored semi-quantitatively (0 to 3+). Oocysts were identified in 29 herds out of 40 (herd prevalence: 72.5%) and the within-herd prevalences ranged from 6 to 100%. The overall calf prevalence was $24.7 \pm 1.8\%$. The prevalence of *Cryptosporidium* sp infection was strongly correlated with calf age with highest values around 45% being observed in 4-14 day-old and 15-30 day-old animals. Furthermore, the highest faecal excretion scores were observed in same age groups. The overall percentage of diarrhoeic calves was 15.0% and peaked to 59.3% for the 4-14 day-old cryptosporidiosis positive animals. In this age class, the diarrhoea frequency was significantly and positively coupled to the intensity of faecal oocyst excretion. These results show that *Cryptosporidium* sp infections are highly prevalent in <1 month-old dairy calves in north-eastern Algeria and should be considered in animals with diarrhoea.

Keywords: Cryptosporidiosis, dairy calves, oocyst excretion, age, diarrhoea, Algeria.

RÉSUMÉ

Prévalence de *Cryptosporidium* sp chez les veaux laitiers dans le nord-est de l'Algérie

La prévalence et l'intensité d'excrétion des oocystes de *Cryptosporidium* sp. ont été déterminées chez des veaux laitiers en Algérie en fonction de leur âge et de leur statut clinique (diarrhée). Des échantillons de fèces ont été fraîchement collectés chez 566 veaux âgés de moins de 12 mois provenant de 40 troupeaux dans 4 régions du nord-est de l'Algérie. Les matières fécales ont été soumises à une technique de concentration puis de coloration par la méthode de Ziehl-Neelsen modifiée et la présence d'oocystes de *Cryptosporidium* sp. a été notée de manière semi-quantitative (0 à 3+). Les oocystes ont été retrouvés dans 29 troupeaux sur 40 (prévalence par troupeau : 72,5 %) avec une prévalence intra-troupeau variant de 6 à 100 %. La prévalence individuelle globale a été de $24,7 \pm 1,8$ %. La prévalence de l'infection à *Cryptosporidium* sp. a été fortement corrélée avec l'âge et les plus fortes proportions d'animaux atteints (environ 45 %) ont été observées chez les veaux de 4-14 jours et ceux de 15-30 jours. En outre, les scores fécaux d'excrétion des oocystes les plus élevés ont aussi été obtenus dans ces 2 classes d'âge. Le pourcentage global de veaux diarrhéiques a été de 15,0 % et la proportion de veaux diarrhéiques a atteint 59,3 % chez les veaux âgés de 4 à 14 jours et positifs pour la cryptosporidiose. Au sein de cette même classe d'âge, la fréquence des diarrhées a été significativement et positivement associée à l'intensité de l'excrétion fécale des oocystes. Ces résultats montrent que les infections à *Cryptosporidium* sp. sont très fréquentes chez les veaux laitiers de moins d'un mois dans le nord-est algérien et doivent être prises en compte dans le contexte de diarrhée néonatale.

Mots clés : Cryptosporidiose, veau laitier, excrétion des oocystes, âge, diarrhée, Algérie.

Introduction

Cryptosporidiosis is a zoonotic protozoan disease of worldwide distribution which affects a large range of vertebrate hosts. Among domestic animals, cattle are considered as one of the most important host for *Cryptosporidium* sp. both in terms of public health as a reservoir for transmission to human beings and of animal disease as this parasite represents a major agent of neonatal diarrhoea of calves [16, 20]. The age of the animals is one of the most important risk factors associated with cryptosporidiosis. Typically, infection occurs at a very early age as it can be detected as soon as 4-5 days of age and lasts for about 3 weeks [18]. Similarly, diarrhoea due to *Cryptosporidium* infection is classically concomitant with high oocyst excretion period, i.e. between 1 and 3 weeks of age [17]. Infection has been associated with economic losses due to the occurrence of the diarrhoea and more rarely to the death of animals [5]. Numerous epidemiological surveys have

been conducted on cattle cryptosporidiosis and most of them concern intensive systems in American or European countries. Algeria is located south of the Mediterranean sea and cattle population is approximately 1.6 million animals, of which 53.55 per cent are dairy cows; the cattle industry is economically important, in particular in the production of milk, which, together with cereals, constitutes a major part of Algerian food production [9]. There is only one published study on *Cryptosporidium* sp occurrence in cattle in Algeria. In a survey on 17 dairy cattle herds, mostly located in center Algeria, KHELIFI *et al.* [8] have shown a mean prevalence of 16.9% in calves aged less than 2 years with higher figures observed in 2- to 3-week-old animals.

The objective of the present study was thus to assess the natural infection in young dairy calves in several regions in north-eastern Algeria through the description of prevalence and intensity of oocyst excretion as well as diarrhoea pattern.

Material and Methods

SAMPLE COLLECTION

The study was carried out in 4 regions (wilaya or department) in eastern Algeria which represents 23% of dairy cattle in Algeria. Forty dairy cattle herds were selected as a convenient sample: 12 from Setif wilaya, 6 from Souk Arras wilaya, 15 from El Tarf wilaya and 7 from Constantine wilaya. Animals were of various dairy cattle breeds mainly local and crossed Friesian breeds. The size of the dairy cattle herd varied from 3 to 58 (mean: 16 ± 15) according to the herd.

During the period between September 2008 and March 2010, faecal samples were collected once from a total of 566 calves aged less than 12 month-old. The animals were divided into four groups according to the age: 1-3 days, 4-14 days, 15-30 days, and 1-12 months. During the visit, all animals corresponding to the defined target (calf < 1 year) were sampled. Faecal samples were taken directly from rectum and identified. Samples were transported directly to the laboratory in a cool box to be stored and processed in the following 3 days. Faeces were classified according to their consistency as diarrhoeic or non-diarrhoeic.

SAMPLE PROCESSING

In the laboratory, faeces were processed according to ALLEN and RIDLEY [1]: a small knob of faeces was diluted in 7 mL of 10% formalin, filtered through gauze, added with 3 mL of ether, shaken vigorously and centrifuged at 1600 g for 1 minute. The sediment was used to prepare thin faecal smear on slide for staining with Ziehl-Neelsen technique modified by HENRIKSEN and POHLENZ [6].

After staining, faecal smears were observed under an optical microscope at 1000 X for identification of oocysts of *Cryptosporidium* sp. The intensity of excretion was evaluated semi-quantitatively according to the average number of oocysts in 30 randomly selected fields. The scoring was as followed: 0: absence of oocyst; +: 1-4 oocysts per field; ++: 5-10 oocysts per field; +++: >10 oocysts per field.

STATISTICAL ANALYSIS

The Chi-square test was used for statistical analysis. The statistical program used was Statmost 2.5 for Windows® (DataMost Corporation, Salt Lake City, USA). Differences were considered as significant when *P* value was less than 0.05.

Results

The number of < 12 month-old calves present in the herd at the time of the visit ranged from 2 to 69. When at least one calf was found excreting *Cryptosporidium* sp. oocysts, the herd was categorized as positive. Twenty-nine herds out of 40 (72.5 ± 7.1 %) were found positive in the four regions studied and the herd prevalence ranged from 53.3% (Setif wilaya) to 100% (Soukh Ahras and Constantine wilayas). For positive farms, the within-herd prevalences ranged from 6 to 100% and the overall prevalence at calf level was 24.7 ± 1.8 % (Table I). The highest individual prevalences were observed in Constantine and El Tarf wilayas (36.8% and 23.3%, respectively).

When considering the oocyst output according to the age of calf, prevalence of infection showed the highest values around 45% in the 4-14 day-old and the 15-30 day-old calf groups when compared to other groups (Table II, *P* < 0.01). Oocyst output was undetectable under 4 days of age and was declining over 1 month of age (18.2%). A significantly higher oocyst excretion scoring (3+) was observed in 4-14 day old and in 15-30 days old animals compared to the following age class (*P* < 0.01 and *P* < 0.05, respectively). Inversely, the proportions of calves showing a mild oocyst excretion (1+) gradually increased according to the calf age.

As far as faecal consistency is concerned, the overall percentage of diarrhoeic calves at the time of sampling was 15.0%. This general figure varied according to the age and showed a higher value (33.3%) for the 4-14 day-old animals compared to the other age classes (*P* < 0.01) and no diarrhoea was recorded in the younger calves (< 3 day old). Additionally, it was found that the majority of infected calves were diarrhoeic in 4-14 day old calves (59.3%) whereas the diarrhoea frequency decreased in positive calves in the other age classes (Table II, *P* < 0.05). Furthermore, diarrhoea occurred more frequently in 4-14 day old calves positive for *Cryptosporidium* showing a maximal oocyst output in faeces (3+) than in those having a lower faecal score (*P* < 0.01) while in the older calves, the majority of diarrhoea cases were recorded in positive animals showing a mild oocyst output (1+).

Discussion

To our knowledge, this work is the first description of *Cryptosporidium* sp in cattle herds in north-eastern Algeria. The herd- and individual prevalences were 72.5% and 24.7%, respectively. These figures were obtained from farms without a deliberate context of diarrhoea and through a unique faecal

Location (wilaya)	Positive farms/total farms	Within-herd prevalence ¹	Individual-calf prevalence
Setif	8/12	12-75%	19.9%
Soukh Ahras	6/6	17-80%	51%
El Tarf	8/15	6-100%	23.3%
Constantine	7/7	6-100%	36.8%
Total	29/40	6-100%	24.7 ± 1.8%

¹for positive farms.

TABLE I: Herd and calf prevalences of *Cryptosporidium* sp in cattle farms in north-eastern Algeria.

	Age classes			
	1-3 days (n = 12)	4-14 days (n = 60)	15-30 days (n = 83)	1-12 months (n = 411)
Overall diarrhoea frequency	0% (0/12)	33.3% (20/60)	22.9% (19/83)	11.2% (46/411)
Cryptosporidiosis individual prevalence	0% (0/12)	45.0% (27/60)	45.8% (38/83)	18.2% (75/411)
diarrhoeic calves/positive calves	0% (0/0)	59.3% (16/27)	39.5% (15/38)	22.7% (17/75)
Oocyst excretion level				
0	100% (12/12)	55.0% (33/60)	54.2% (45/83)	81.8% (336/411)
diarrhoeic calves/negative calves	0% (0/12)	12.1% (4/33)	8.9% (4/45)	8.6% (29/336)
1+	0% (0/12)	18.5% (5/27)	44.7% (17/38)	78.7% (59/75)
positive(1+) calves/diarrhoeic calves	-	6.3% (1/16)	40.0% (6/15)	70.6% (12/17)
2+	0% (0/12)	29.6% (8/27)	31.6% (12/38)	10.7% (8/75)
positive(2+) calves/diarrhoeic calves	-	31.3% (5/16)	33.3% (5/15)	23.5% (4/17)
3+	0% (0/12)	51.9% (14/27)	23.7% (9/38)	10.7% (8/75)
positive(3+) calves/diarrhoeic calves	-	62.5% (10/16)	26.7% (4/15)	5.9% (1/17)

Scoring of the oocyst excretion level: 0: absence of oocyst; +: 1-4 oocysts per field; ++: 5-10 oocysts per field; +++: >10 oocysts per field.

TABLE II: *Cryptosporidium* sp infection in calves in north-eastern Algeria: individual prevalence, intensity of oocyst excretion and frequencies of diarrhoea according to the age of calves (n = 566).

sampling of <12 month-old calves. These results could have been substantially increased from farms with history of neonatal enteritis and with repeated faecal sampling of the same animals giving eventually a cumulative incidence [15, 19]. In central Algeria, a lower individual prevalence of 16.97% was observed [8] but the results were obtained from a limited sampling of 12 cattle farms and without concentration procedure prior to faecal smear staining. In neighbouring countries, prevalence of infection was 30.2 % in dairy calves less than 6 week old in Egypt [2] and 45.2 % in calves less than 3 week old in Morocco.

Higher frequency of oocyst output was seen in 4-14 day-old and 15-30 day-old calf groups (around 45 %) what is a steady and characteristic trait of *Cryptosporidium* sp infection in young cattle [4, 11]. When calves are getting older, infection persists in most of the animals but is normally no longer detectable at a high occurrence through routine faeces examination like faecal smears. When sensitive techniques are processed (concentration techniques and/or direct immunofluorescence), oocyst excretion may be detected at a relatively high occurrence. In our study, prevalence of *Cryptosporidium* sp infection was 18.2% in > 1 month-old calves which is consistent with the results of studies performed in older calves in North America and Europe [3, 7, 14]. Levels of oocyst excretions, assessed semi-quantitatively, were higher between 4 and 30 days of age and were related to a higher proportion of diarrhoeic faeces. Such positive relationship between diarrhoea and *Cryptosporidium* oocyst output has been described in calves with simultaneous occurrence of diarrhoea and high oocyst counts [10, 13, 21]. However, calf neonatal diarrhoea in the field is a more complex and multifactorial disease that involves *Cryptosporidium* and other pathogens exposure as well as environmental and management factors [12].

Our study confirms the frequent occurrence of *Cryptosporidium* sp in diarrhoeic calves in Algeria. As several species and/or genotypes may be involved according to the different age classes of cattle [22], further studies are needed to have a

better understanding of *Cryptosporidium* infection in cattle farms and its zoonotic potential.

References

1. - ALLEN A.V.H., RIDLEY D.S.: Further observations on the formol ether concentration technique for faecal parasites. *J. Clin. Pathol.*, 1970, **23**, 545-546.
2. - AMER S., HONMA H., IKARASHI M., TADA C., FUKUDA Y., SUYAMA Y., NAKAI Y.: *Cryptosporidium* genotypes and subtypes in dairy calves in Egypt. *Vet. Parasitol.*, 2010, **169**, 382-386.
3. - CASTRO-HERMIDA J.A., CARRO-CORRAL C., GONZALEZ-WARLETTA M., MEZO M.: Prevalence and intensity of infection of *Cryptosporidium* spp and *Giardia duodenalis* in dairy cattle in Galicia (NW Spain). *J. Vet. Med. B*, 2006, **53**, 244-246.
4. - CASTRO-HERMIDA J.A., GONZALEZ-LOSADA Y.A., MEZO-MENENDEZ M., ARES-MAZAS E.: A study of cryptosporidiosis in a cohort of neonatal calves. *Vet. Parasitol.*, 2002, **106**, 11-17.
5. - De GRAAF D.C., VANOPDENBOSH E., ORTEGA-MORA L.M., ABBASSI H., PEETERS J.E.: A review of the importance of cryptosporidiosis in farm animals. *Int. J. Parasitol.*, 1999, **29**, 1269-1287.
6. - HENRIKSEN S.A., POHLENZ J.: Staining of cryptosporidia by a modified Ziehl-Neelsen technique. *Acta Vet. Scand.*, 1981, **22**, 594-596.
7. - HUETINK R.E., VAN DER GIESSEN J.W., NOORDHUIZEN J.P., PLOEGER H.W.: Epidemiology of *Cryptosporidium* spp. and *Giardia duodenalis* on a dairy farm. *Vet. Parasitol.*, 2001, **102**, 53-67.
8. - KHELEF D., SAIB M.Z., AKAM A., KAIDI R., CHIRILA V., COZMA V., ADJOU K.T.: Epidémiologie de la cryptosporidiose chez les bovins en Algérie. *Rev. Méd. Vét.*, 2007, **158**, 260-264.
9. - MADR, Ministère Agriculture et du Développement Rural Rapport sur la situation du secteur agricole. République Algérienne Démocratique et Populaire, Alger, Algérie, 2005, <http://www.minagri.dz/pdf/Rapports/Rapport%20sur%20la%20situation%20du%20secteur%20agricole%202005.pdf>.
10. - Mc CLUSKEY B.J., GREINER E.C., DONOVAN G.A.: Patterns of *Cryptosporidium* oocysts shedding in calves and a comparison of two diagnostic methods. *Vet. Parasitol.*, 1995, **60**, 185-190.
11. - NYDAM D.V., WADE S.E., SCHAFF S.L., MOHAMED H.O.: Number of *Cryptosporidium parvum* oocysts or *Giardia* spp cysts shed by dairy calves after natural infection. *Am. J. Vet. Res.*, 2001, **62**, 1612-1615.
12. - O'HANDLEY R.M.: *Cryptosporidium parvum* infection in cattle: are current perceptions accurate? *Trends Parasitol.*, 2007, **23**, 477-480.

13. - O'HANDLEY R.M., COCKWILL C., Mc ALLISTER T.A., JELINSKI M., MORCK D.W., OLSON M.E.: Duration of naturally acquired giardiasis and cryptosporidiosis in dairy calves and their association with diarrhea. *J. Am. Vet. Med. Assoc.*, 1999, **214**, 391-396.
14. - OLSON M.E., GUSELLE N.J., O'HANDLEY R.M., SWIFT M.L., Mc ALLISTER T.M., JELINSKI M.D., MORCK D.W.: *Giardia* and *Cryptosporidium* in dairy calves in British Columbia. *Can. Vet. J.*, 1997, **38**, 703-706.
15. - SOLTANE R., GUYOT K., DEI-CAS E., AYADI A.: *Cryptosporidium parvum* (Eucoccidiorida: Cryptosporiidae) in calves: results of a longitudinal study in a dairy farm in Sfax, Tunisia. *Parasite*, 2007, **14**, 309-312.
16. - THOMPSON R.C., PALMER C.S., O'HANDLEY R.: The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. *Vet. J.*, 2008, **177**, 18-25.
17. - TROTZ-WILLIAMS L.A., JARVIE B.D., MARTIN S.W., LESLIE K.E., PEREGRINE A.S.: Prevalence of *Cryptosporidium parvum* infection in southwestern Ontario and its association with diarrhea in neonatal dairy calves. *Can. Vet. J.*, 2005, **46**, 349-351.
18. - TROTZ-WILLIAMS L.A., WAYNE MARTIN S., LESLIE K.E., DUFFIELD T., NYDAM D.V., PEREGRINE A.S.: Calf-level risk factors for neonatal diarrhea and shedding of *Cryptosporidium parvum* in Ontario dairy calves. *Prev. Vet. Med.*, 2007, **82**, 12-28.
19. - UGA S., MATSUO J., KONO E., KIMURA K., INOUE M., RAI S.K., ONO K.: Prevalence of *Cryptosporidium parvum* infection and pattern of oocyst shedding in calves in Japan. *Vet. Parasitol.*, 2000, **94**, 27-32.
20. - XIAO L.: Molecular epidemiology of cryptosporidiosis: an update. *Exp. Parasitol.*, 2010, **124**, 80-89.
21. - XIAO L., HERD R.P.: Infection patterns of *Cryptosporidium* and *Giardia* in calves. *Vet. Parasitol.*, 1994, **55**, 257-262.
22. - XIAO L., RYAN U.M.: Molecular epidemiology. In: *Cryptosporidium* and Cryptosporidiosis, Fayer R. and Xiao L. (eds), 2nd edition, Boca Raton, FL, USA, CRC Press, 2008, pp.: 119-163.