

## Full Length Research Paper

# Study of *Giardia* spp., *Cryptosporidium* spp. and *Eimeria* spp. infections in dairy cattle in Algeria

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The prevalence of *Giardia* spp., *Cryptosporidium* spp and *Eimeria* spp. in dairy cattle was studied on sixteen farms in three regions at north-eastern Algeria. 690 fecal samples were collected (330 cows and 360 calves aged 1 day to 12 months), were processed by a concentration technique (to examine them for *Giardia* spp. cysts and *Eimeria* spp. oocysts), followed by a modified Ziehl-Neelsen staining to examine them for *Cryptosporidium* spp. oocysts. *Giardia* spp., *Cryptosporidium* spp. and *Eimeria* spp. were reported in 50.75 and 62.5% of all farms, respectively. The prevalence of *Giardia* spp. in calves and adults was 14.72 and 11.81%, respectively with a high level in calves aged 4 to 12 months compared to other age groups ( $p < 0.05$ ). *Cryptosporidium* spp. was observed principally in calves 4 to 30 days old compared to other classes age of calves ( $p < 0.01$ ) and the overall prevalence was 26.11% in calves and 18.78% in adults. *Eimeria* spp. oocysts were excreted by 12.77% of the calves and 4.54% of adults. *Giardia* spp. and *Cryptosporidium* spp. were detected more frequently in diarrheic calves than in non-diarrheic calves ( $p < 0.01$ ). The results demonstrated the presence of these parasites in various age groups of dairy cattle in Algeria which could have important economic and welfare implications.

**Key words:** *Giardia* spp., *Cryptosporidium* spp., *Eimeria* spp., diarrhea, calves, cows, Algeria.

## INTRODUCTION

*Giardia* spp, *Cryptosporidium* spp and *Eimeria* spp are intestinal protozoan parasites affecting cattle and many vertebrates. Their transmission is essentially by fecal-oral route (Cacciò et al., 2006; Mage and Reynal, 2003). Giardiasis is a zoonosis parasite caused by *Giardia duodenalis* in cattle. It manifests clinically by diarrhea, stunting and wasting (Le Drean-Quenech'du, 2003). The excretion of cysts in feces may last several weeks unlike *Cryptosporidium parvum*, *Giardia duodenalis* affects older calves (O'Handley et al., 1999). Cryptosporidiosis is

mainly due to *C. parvum*. This species is a zoonosis and a dominant digestive tract pathogen in young ruminants. The principal characteristic of this parasitic infection is the large infectivity and the great difficulty of treatment or prevention in the absence of truly effective medicaments. During the first two weeks of life, the calf can shed thousands of oocysts of *C. parvum*, while adult individuals remain mostly asymptomatic carriers (Chartier and Paraud, 2010).

*Eimeria bovis* and *Eimeria zuernii* are considered to be

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the most pathogenic species in cattle. After infection, the appearance of the diarrhea usually begins after 2 weeks. The disease usually occurs in older calves from 3 weeks to 18 months (Mage and Reynal, 2003). Several studies in the world determined the prevalence of infection of these three parasites in cattle (O'Handley et al., 1999; Fayer et al., 2000; Gül et al., 2008; Huetink et al., 2001). However, in Algeria the frequency of these three protozoa in cattle is poorly understood, especially for *Giardia* spp. and *Eimeria* spp. This study is a contribution to the study of the epidemiology of these three protozoa in dairy cattle in three regions (Bejaia, Setif and Souk Ahras) in Algeria.

## MATERIALS AND METHODS

### Study area

This study was realized in three areas in North-eastern Algeria: Bejaia, Setif and Souk Ahras.

### Animals and samples

During a period of January, 2010 to December, 2010, a study was conducted in 16 dairy farms selected in a random manner. Cattle were the only species bred in these farms. A single fecal sample was collected from each animal. A total of 690 fecal samples were collected including 360 calves (aged one day to 12 months) and 330 cows (adults > 24 months). Samples of 47 calves were diarrheal and no adult was found with diarrhea (liquid faeces). The number of samples in the region of Bejaia was 223 (99 calves and 124 cows) realized in 5 farms. In the Setif region, the number was 407 samples (201 calves and 206 cows) belong to 8 farms. At the region of Souk Ahras, this number was 60 calves, belonging to 3 farms. These samples were collected in sterile vials directly from the rectum of animals (or digital rectal in younger animals) and sent to the laboratory in a cooler (at a temperature of 4°C approximately) for a period of one to six hours for analyses of suspected parasites.

### Laboratory analysis

The samples were analyzed directly upon their arrival at the laboratory. Each sample was analyzed by the technique of Ritchie simplified by Allen and Ridley (1970). A drop of the sediment was placed between slide and cover slide to search *Giardia* spp. and *Eimeria* spp. The examination was conducted under an optical microscope (200 × 400). To search for *Cryptosporidium* spp. a fecal smear was prepared from the sediment obtained by the method previously described and then the smear was stained by the technique of Ziehl-Neelsen modified by Henriksen and Pohlenz (1981). The examination was conducted under an optical microscope (400 × 800).

### Statistical analysis

The  $\chi^2$  test (95% confidence interval) was used to study the influence of the age of the calves and the diarrhea on the prevalence excretion of *Giardia* spp. *Cryptosporidium* spp. and *Eimeria* spp. The same test was also used to investigate the influence of different types of infections (singles-infections or

associations) of these three parasites on the prevalence excretion (significant results when  $p < 0.05$ ).

## RESULTS

In all sixteen dairy farms concerned by this study *Giardia* spp., *Cryptosporidium* spp. and *Eimeria* spp. were found in 8 (50%), 12 (75%) and 10 farms (62.50%), respectively. With calves aged between 1 day to 12 months, the search for *Giardia* spp revealed that 14.72% (53/360; 95% confidence interval) subjects were excreting. This prevalence varied in different farms from 12.12 to 28.88%. The *Cryptosporidium* spp. prevalence in the different farms varied from 15.78 to 42.22% with the overall of 26.11% (94/360; 95% confidence interval). By contrast, overall *Eimeria* spp. prevalence in calves was the lowest at 12.77% (46/360; 95% confidence interval) (with a range of prevalence on different farms from 5.55 to 27.77%). In adult cows, 11.81% (39/330; 95% confidence interval), 18.78% (62/330; 95% confidence interval) and 4.54% (15/330; 95% confidence interval) were excreting *Giardia* spp, *Cryptosporidium* spp. and *Eimeria* spp., respectively (Table 1). In different farms, the prevalence varied from 2.08 to 25.68% for *Giardia* spp, 7.14 to 50% for *Cryptosporidium* spp and 8.33 to 20.83% for *Eimeria* spp.

*Giardia* spp., *Cryptosporidium* spp. and *Eimeria* spp. were found in calves after 8, 4 and 12 days old, respectively and in adults (Table 1). The study of the relationship between the animal age and the prevalence of *Giardia* spp. showed that calves aged between 4 to 12 months were the most affected (25.86%; 30/116; 95% confidence interval) compared to all others age groups ( $p < 0.05$ ). Calves aged 4 to 30 days (36.70%; 51/139; 95% confidence interval) have a higher prevalence of *Cryptosporidium* spp. compared to other age groups ( $p < 0.01$ ) and calves aged 1 to 12 months (15.71%; 33/210; 95% confidence interval) have been the most excretory of *Eimeria* spp. cysts ( $p < 0.05$ ).

*Giardia* spp., *Cryptosporidium* spp. and *Eimeria* spp. were demonstrated in 36.17% (17/47; 95% confidence interval), 68.08% (32/47; 95% confidence interval) and 19.14% (9/47; 95% confidence interval) of the diarrheal calves, respectively (Table 2). The presence of *Giardia* spp. and *Cryptosporidium* spp. in calve samples was significantly associated with diarrhea ( $p < 0.01$ ). By contrast, no significant relationship was observed between the excretion of *Eimeria* spp. and diarrhea. Also, diarrheic calves aged 4 to 30 days were the most excretory of *Cryptosporidium* spp. oocysts with a prevalence of 88% ( $p < 0.01$ ) against a prevalence of 45.45% among those aged from 1 to 12 months. Of the 47 diarrheic calves, 46.80% were revealed excreting oocysts of *Cryptosporidium* spp. without the presence of *Giardia* spp. and *Eimeria* spp. This prevalence was significantly high compared to other types of associations ( $p < 0.01$ ).

**Table 1.** Prevalence of *Giardia* spp, *Cryptosporidium* spp and *Eimeria* spp infections in dairy cattle according to age in three areas in North-eastern Algeria: Bejaia, Setif and Souk Ahras.

Age	No. of sample	Number of animals infected (%; 95% confidence interval)		
		<i>Giardia</i> spp.	<i>Cryptosporidium</i> spp.	<i>Eimeria</i> spp.
1-3 days	11	0 (-)	0 (-)	0 (-)
4-7 days	23	0 (-)	8 (34.78)	0 (-)
8-14 days	35	3 (8.57)	11 (31.42)	3 (8.57)
15-21 days	39	3 (7.69)	15 (38.46)	5 (12.82)
22-30 days	42	4 (9.52)	17 (40.47)	5 (11.9)
1-3 months	94	13 (13.82)	22 (23.40)	16 (17.02)
4-12 months	116	30 (25.86)	21 (18.10)	17 (14.65)
Adults	330	39 (11.81)	62 (18.78)	15 (4.54)

**Table 2.** Prevalence of *Giardia* spp., *Cryptosporidium* spp. and *Eimeria* spp. infections in diarrheic and non-diarrheic calves (aged 01 day to 12 months).

Aspect of the feces	No. of calves infected (%)		
	<i>Giardia</i> spp.	<i>Cryptosporidium</i> spp.	<i>Eimeria</i> spp.
Diarrheal feces (n=47)	17 (36.17)	32 (68.08)	9 (19.14)
Non diarrheal feces (n=313)	36 (11.5)	62 (19.8)	37 (11.82)
Total (n=360)	53 (14.72)	94 (26.11)	46 (12.77)

**Table 3.** Prevalence of these three parasites infections in diarrheic calves (aged 1 day to 12 months) according to the types of infections.

Infection type (single-infection or association)	No. of diarrheal samples	%
<i>Cryptosporidium</i> spp. only	22	46.80
<i>Giardia</i> spp. only	9	19.14
<i>Eimeria</i> spp. only	0	0
<i>Cryptosporidium</i> spp. + <i>Giardia</i> spp.	4	08.51
<i>Cryptosporidium</i> spp. + <i>Eimeria</i> spp.	5	10.63
<i>Giardia</i> spp. + <i>Eimeria</i> spp.	3	6.38
<i>Cryptosporidium</i> spp. + <i>Giardia</i> spp. + <i>Eimeria</i> spp.	1	02.12

Thus, only three calves (6.38%) excreted cysts of *Giardia* spp. and *Eimeria* spp. alone. In addition, a single calf (2.12%) was reported excretory of these three parasites at the same time (Table 3).

## DISCUSSION

Several studies in the world determine the prevalence of these three parasites in cattle farms (O'Handley et al., 1999; Fayer et al., 2000; Gül et al., 2008; Huetink et al., 2001). The results reached by these authors show widely varying rates, some of which are consistent with our observations. For *Giardia* spp., Ruest et al. (1998) reported

that 45.7% of all dairy farms were positive. Quílez et al. (1996), on their part, found that 53.3% of farms are infected with *Giardia* spp. These results are close to ours (50%) concerning *Cryptosporidium* spp., Olson et al. (1997) reported that 80% of farms were infected and according to Quílez et al. (1996), this prevalence was 63.3%. In our study, *Cryptosporidium* spp. was observed in 75% of all farms. Concerning *Eimeria* spp., we reported that 62.5% of farms were infected and the results obtained by different studies report that the infection prevalence had been ranging from 43.2 to 100% of the farms tested (Bourguignon et al., 2010; Lassen et al., 2009; Richard, 2006).

In cattle, the prevalence of *Giardia* spp. can range from

9 to 73% (Olson et al., 1997; McAllister et al., 2005). In our study this prevalence is 14.72% in calves (aged 1 day to 12 months) and 11.81% in adults. These proportions are close to the results presented by Gow and Waldner (2006) on the calves (22.6%) and adults (17%). Again, Quílez et al. (1996) reported that the prevalence in calves was 18.23 and 2.2% in adults.

The excretion of *Giardia* spp. in calves can begin on the fourth day after birth (Wade et al., 2000). In this study, *Giardia* spp. was observed at the age of 8 days, which is similar to the results highlighted by Bjorkman et al. (2003) which reported that excretion begins at the age of nine days. We noted in this study also that the highest *Giardia* spp. prevalence was reported in calves aged 4 to 12 months ( $p < 0.05$ ). A study showed that the highest prevalence was observed in calves aged 4 to 5 months (Huetink et al., 2001).

On the other hand, we found that the prevalence of *Cryptosporidium* spp. in calves and adults was, respectively 26.11 and 18.78%. In Algeria, the prevalence of cryptosporidiosis in cattle is poorly understood. The studies focused in the area of Mitidja, revealing the excretion rate of *Cryptosporidium* spp. in calves ranging from 21.21 to 28.44% (Akam et al., 2004; Khelef et al., 2007; Khelef et al., 2002). Quílez et al. (1996) in a study conducted in Spain, reported that the prevalence in calves from 3 days to 24 months of age is 20.97% and in adults is 17.8%. These results are similar to ours.

The relatively high prevalence (18.78%) recorded during our study in adults is due to the fact that these animals are cows from calving, and in a very contaminated environment with *Cryptosporidium* spp. The same observation was established by other authors (Huetink et al., 2001; Quílez et al., 1996; Olson et al., 1997). After the detection of *Cryptosporidium* spp. in the feces of calves of 4 days of age, we found that the calves were infected immediately after birth. This is consistent with the studies of several authors (Quílez et al., 1996; Castro-Hermida et al., 2002).

In addition, we noted that the highest rate of the excretion was observed in calves aged 4 to 30 days ( $p < 0.01$ ). Such remark confirms the observation noted by other authors (Huetink et al., 2001; Olson et al., 1997). The prevalence of bovine coccidiosis varies from 3.1 to 31.3% depending on the species involved (Kennedy and Kralka, 1987). In this study, *Eimeria* spp. was isolated from 12.77% of all calves. Thus, calves aged 1 to 12 months have been the most infected ( $p < 0.05$ ). This result was supported by Hansen and Perry (1994) in calves of this age group. Oocysts of *Eimeria* spp. were observed in our study from the twelfth day of age. This result corroborates the observation from Chibunda et al. (1997).

On the other hand, we noted that the prevalence of *Eimeria* spp. is 4.54% for adults a result quite similar to that reported by Fayer et al. (2000) which is 5.26%. Of

the 360 calves concerned by this study, 47 (13.05%) of them had diarrhea and 36.17% of these diarrheal calves excreted *Giardia* spp. and this excretion was significantly associated with diarrhea ( $p < 0.01$ ). This result is identical to that obtained by McAllister et al. (2005) who showed that 36% of cases of giardiasis in diarrheic calves and excretion of *Giardia* spp. was significantly associated with diarrhea. But some authors have observed that the excretion of *Giardia* spp. in calves has no relationship with diarrhea (Huetink et al., 2001; Quílez et al., 1996; Adjou, 2011).

Cryptosporidiosis has a major role in neonatal diarrhea calves (Chartier and Paraud, 2010). The immune system of calves during the first days of life is still immature, hence the high sensitivity to cryptosporidiosis. Several authors have noted a higher prevalence of the parasite in the feces of diarrheic calves (Huetink et al., 2001; Quílez et al., 1996; Adjou, 2011). In our study, *Cryptosporidium* spp. was more observed in diarrheic calves than in those who were not and especially for those aged less than 30 days ( $p < 0.01$ ). The prevalence reported in diarrheal and non-diarrheal calves are 68.08 and 19.08%, respectively. These values are similar to those observed by Sevinc et al. (2003), who found 63.92 and 9.85% in diarrheal and non-diarrheal calves, respectively.

For *Eimeria* spp. we found that 19.14% of diarrheic calves were excreting, but no significant relationship was observed with the presence or absence of diarrhea. The same observation was reported by Lassen et al. (2009) and Quílez et al. (1996). However, Alemayehu et al. (2013) in Ethiopia and Chibunda et al. (1997) in Tanzania noted that the excretion of *Eimeria* spp. was very significantly associated with diarrhea. The study of the association between these three parasites in calves shows that diarrheal calves tend to excrete *Cryptosporidium* spp. alone than in combination with the other two parasites ( $p < 0.01$ ). The same observation was represented by Adjou (2011) and Quílez et al. (1996).

## Conclusion

From this study, we conclude that infection of cattle in Algeria by these three protozoa seems very important. These parasites were observed in young calves and adults. Adult cows asymptomatic porters are important sources of infections. *Cryptosporidium* spp. mainly affects young calves under 30 days of age. However, *Giardia* spp. and *Eimeria* spp. hit older calves, 4 to 12 months and 1 to 12 months, respectively. The excretion of *Cryptosporidium* spp. and *Giardia* spp. was significantly related to the presence of diarrhea.

## Conflict of Interest

The authors declare that they have no conflict of interests.

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