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Prevalence of Cryptosporidium Oocysts in Calves in Two Aereas from Aestern Algeria

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Abstact: The study was conducted in two regions in Algeria eastern (Annaba, Eltarf) and undertaken from September 2015 to May 2016 to determine the prevalence of *Cryptosporidium* infection. Faecal samples from 214 calves were collected and examined for the identification of *Cryptosporidium* spp. oocysts by using the Ziehl–Neelsen modified technique in 58 (27.10%) calves. No significant difference in prevalence was observed between the two areas (P>0.05). Calves were grouped according to their age as follows: 1-15, 16-30 and 31-61days and Cryptosporidium infection was detected in 56.25%, 24.24% and 16%, of the calves in the age groups, respectively.A significant age-associated decrease in the detection rate of *Cryptosporidium* infection (P = 0.0335 ; P<0.05) was found. No significant relationship between infection to protozoan and sex of studied calves (P= 0.1667).Cryptosporidium spp oocysts were detected in 38.75% of 80 diarrheic and 20.14% of 134 non-diarrheic stools.No significant association was observed between diarrhea and the presence of *Cryptosporidium* sp.oocysts (P = 0.3761; P>0.05).The findings of this study indicate that *Cryptosporidium* is commonly found among 1-30 days calves and it has association with age calves and demonstrated its importance in eastern Algeria.

Key words: Prevalence · *Cryptosporidium* spp · Oocyst · Calves · Diarrhea · Aestern Algeria

INTRODUCTION

Cryptosporidiosis is an acute or chronic enteric disease of young or immunosuppressed animals and humans caused by Cryptosporidium species in the phylum Apicomplexa.Cryptosporidium species are the important intestinal protozoans of domestic animals. With at least two distinct species namely *Cryptosporidium* parvum and Cryptosporidium andersoni[1].Most *Cryptosporidium* infections in pre-weaned calves were due to *C.parvum* [2]. The first description of *Cryptosporidium* was given by Ernest Edward [3] who isolated it in the peptic glands of laboratory mice mouse. Being zoonotic, infected animals pose health risk to humans especially in the immunocompromised individuals like people living with HIV/AIDS [4].

In many developed countries, only a few or no parasitic protozoa are included in operational surveillance systems, as the major focus is on bacterial and viral infections [5,6].However, evidence suggests that while some enteric protozoa, such as *Cryptosporidium* is isolated frequently from diarrheal humans and animals in developing regions such as Asia and sub-Saharan Africa [7-8].

Cryptosporidiosis is the clinical disease, usually presenting as a gastro-enteritis-like syndrome ranging from mild to severe symptoms depending on the site of infection, nutritional and immune status of the host [9]**.** Calves are primarily infected via the fecal-oral route and it takes less than 50 oocysts to infect a healthy calf [10]**.** Clinically, the disease is characterized by anorexia and diarrhea, often intermittent, which may result in poor growth rate [11-12]. Yet it has been incriminated as an important cause of diarrhea in neonates [13-14].

According to [15], although calves 1-3 weeks old seem to be most susceptible, *Cryptosporidium spp.*was one of the most frequent pathogens responsible for outbreaks of severe diarrhea, mainly in calves up to one

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month of age [16-17]. The adult animals are generally refractory to infection,infected animals can act as asymptomatic carriers and shed large numbers of oocysts into the environment and remain a main source of infection to other domestic and wild animals [18]. However, most of the published studies are from North America,India, Europe and Japan, Cryptosporidiosis in cattle has been reported from different parts of the world with approximately 45.5% incidence in USA, 24.5% in UK, 26% in USSR, 40% in Germany and 27% in Hungary [19]. Different estimations of prevalence of *Cryptosporidium* spp. were reported in cattle ranged from 10.24% to 37.50% [20-24] in different countries.

Little informations has been published about calves cryptosporidiosis in African countries including Algeria and epidemiological studies have not yet been conducted on a wide scale and in large number of animals.The only documented report of *Cryptosporidium* infection in Algeria is that [25], who reported 16,97% prevalence in calves after a épidémiologique study conducted to determine prevalence in catalle in aestern and center of Algeria.In light of the veterinary importance, causation of production losses and its zoonotical potential, more knowledge about the prevalence of the parasite was needed.

Therefore, the main objective of the present study was to determine the prevalence of cryptosporidiosis in calves up to two months of age in two communities in eastern Algéria and to analyze the association between the prevalence rate with age, sex and diarrhoea for the suggestion of its better control strategies.

MATERIALS AND METHODS

Animals and Faecal Sample Collection:
The study was conducted in two eastern areas (Annaba, Eltarf) of Algeria, from September 2015 to May 2016. The farms participating in this study had been selected randomly.Each farm included in this survey were visited once.

A total of 214 faecal samples (96 female and 118 male) were collected directly from the rectum of calves (birth to 60 days old) from two eastern localities of Algeria (Annaba, Eltarf), using sterile plastic gloves and placed in technically sterile,suitable leak-proof plastic containers containers, tightly closed and labeled. These calves were grouped into three different age groups: 1-15 days (n=32), 16-30 days (n=132) and 31-60 days (n=50).

Specimens were stored in a refrigerator at +4C and quickly transported after collection to the Laboratory (Parasitological laboratory IBN SINA Hopitale Annaba). The data related to area, age, sex and consistency of faeces (diarrhoeic or non- diarrhoeic) was collected from each animal through a questionnaire.

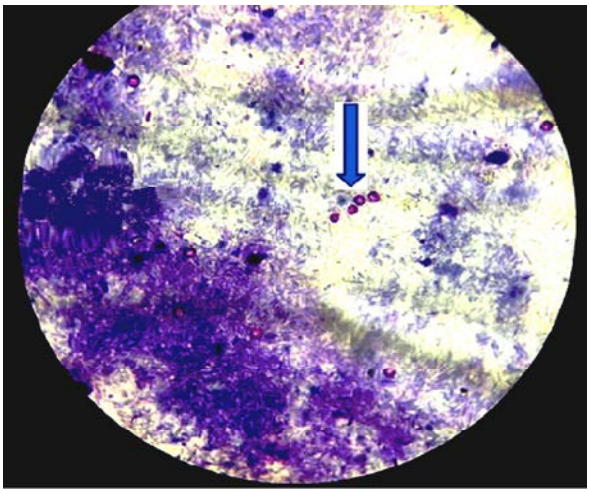

Fig. 1: Cryptosporidium oocysts observed in fecal smears of calves (X100).

Parasitological Examination of Faecal Smears: For direct faecal smear examination, a thin faecal smear was prepared with the help of a sterilized ear bud on a clean microscopic glass slide and air dried. After fixation by methanol for 5minutes.Here, the presence of Cryptosporidium spp.oocysts in fecal smears was detected using the modified Ziehl-Neelsen staining technique as described by [25].

In brief, after fixation and air dried, smears were stained with Carbol Fuchsin solution for 1 heurs and rinsed thoroughly in tap water. Then decolorization was done in 2% acid sulfirique for 20s and again the smears were rinsed with tap water and then, the smears were counterstained with 0.5 % Malachite Green for 5 minutes.The smears were finally washed in tap water, air-dried and were examined.

The stained smears were observed under microscope with a ×100. The Cryptosporidium spp. oocysts were visualized as densly stained red round bodies clearly distinguishable against a green background. Cryptosporidium infection was scored positive if at least one morphologically distinct Cryptosporidium spp.oocyst was observed.Only those which were positive on modified Zeihl-Neelson technique were recognized as positive and others were registered as negative.

Data Analysis: Data collated at the end of the study were subjected to statistical analysis using R logiciel. Prevalence rates were calculated by dividing the numbers

of infected individuals by the total number of individuals examined and expressed as percentages.Statistical analyses were performed using the chi-square test to determine the association of the disease with some risk factors and significance was considered when P-value is less than 0.05.

RESULTS

Prevalence of Cryptosporidium Spp: In this study, fecal samples were collected from 214 calves for two eastern areas of Algeria (Annaba, Eltarf) and examined using Staining of Cryptosporidia by modified Ziehl- Neelsen technique. The examination showed that 27.10% (58/214) had forms identified as Cryptosporidium spp. Of the 88 samples analyzed belonging Eltarf, 26 showed oocysts (29.5%), while of the 126 samples of Annaba analyzed 32 were positive, revealing a prevalence of 25.3%.No differences were observed statistically significant between infection and study area (Annaba, Eltarf) (P =0.8792 P > 0.05). (Table 1)

When the prevalence of Cryptosporidiun spp,was analysed by age, it was observed that calves between the age 1-15 days had a higher prevalence (56,25%).This was followed by (24 ,24%) among 16-30 days while the lowest 16% was recorded among 30-60 days. (Table 2).

Consequently,there was a significant difference in the prevalence of infection between the three age groups (P= 0.0335 P<0.05). The *Cryptosporidium* infection rate was significantly (P<0.05) higher in calves aged 1-15 days than in those aged 16-60 days.The *Cryptosporidium* infection rate was also significantly (P<0.05) higher in calves aged 16-30 days than 31-60 days old calves (Table 2).

A sex prevalence was found in the present study with the prevalence rate of 18,70% and 33,80% in female and male respectively (Table 3). Statistical analysis showed no significant relationship between infection to protozoan and sex of studied calves (P= 0.1667 P > 0.05).

Table 1: The infection rate of *Cryptosporidium* spp.in calves in two eastern regions of Algeria

Regions	No. of animals examined	*Cryptosporidium* spp. No.Positive	*Cryptosporidium* spp. Percentage%
Annaba	126	32	25,39%
Eltarf	88	26	29.5%
Total	214	58	27 ,10%
Chi-square value = 0.25744		P value = 0.8792	

Prevalence of *Cryptosporidium* infection in diarrheic and non-diarrheic calves is shown in (Table 4).

An examination of the consistency of the 214 fecal samples showed that 80 of samples were diarrheic 37,38% (80/214).Of these 38,75% (31/80) tested positive for *Cryptosporidium* oocysts. The remaining were non-diarrheic (134/214) 62,61%, with 20,14% (27/134) of these testing positive (Table 4).

On the other hand, study on the diarrheic history of calves showed that this factor can not be cause for infection to Cryptosporidium and there is no significant relationship between this factor and infection to protozoan (With diarrhoea Chi-square valu =3.1024 Pvalue = 0.3761) and (Without diarrhoea Chi-square value = 6.6289 P value = 0.08471 P > 0.05) (Table 4).

The prevalence of infection peaked among calves between 1-15 days in both diarrheic and without diarrhoea groups withe (64,28%) and (50%) respectively The lowest prevalence of *Cryptosporidium* was observed in claves of 30-60 days in non diarrhoeic (11,53%). Showed that this factor can not be cause for infection to Cryptosporidium and there is no significant relationship between this factor and infection to protozoan (P > 0.05).

Table 2: Prevalence of Cryptosporidium in calves, with age group in two eastern areas of Algeria

Age range (Days)	Areas: Eltarf Number of animals examined	Eltarf Positive cases (%)	Annaba Number of animals examined	Annaba Positive cases (%)	Totale Number of animals examined	Totale Positive cases (%)
1-15 days	6	4 (66,66)	26	14 (53,24)	32	18 (56,25)
16-30 days	64	18 (28,12)	68	14 (20,58)	132	32 (24,24)
31-60 days	18	4 (22,22)	32	4 (12,50)	50	8 (16)
Total	88	26 (29,54)	126	32 (25,39)	214	58 (27,10)
Chi-square value = 8.7008		P value = 0.0335 P< 0.05				

Table 3: The prevalence rate of *Cryptosporidium* in calves according to the sex in two eastern communities of Algeria

	Areas								
	Eltarf			Annaba			Totale		
		Positive			Positive			Positive	
Sex	N studied	N positive	Percentage (%)	N studied	N positive	Percentage (%)	N studied	N positive	Percentage (%)
Female	32	6	18.7	64	12	18.7	96	18	18.70
Male	56	20	35.7	62	20	32.2	118	40	33.80
Total	88	26	29.5	126	32	25.3	214	58	27.10

N:number of calves

Eltarf Chi-square value = 1.5944 P value = 0.4506 P > 0.05

Annaba Chi-square value = 1.8065 P value = 0.4052 P > 0.05

Total Chi-square value = 3.5826 P value = 0.1667 P > 0.05

Table 4: Prevalence of *Cryptosporidium* oocyst with age group and diarrheal status of calves in two eastern areas of Algéria (ELTARF and ANNABA)

			Category Diarrhoea Status			
			With diarrhoea		Without diarrhoea	
Age groups Days	Numbers of Examined calves	Numbers of positve calves	Examined	Positive%	Examined	Positive%
1-15 days	32	18	14	9 64.28%	18	9 50%
16-30 days	132	32	42	17 40.47%	90	15 16.66%
31-60 days	50	8	24	5 20.83%	26	3 11.53%
Total	214	58	80	31 38.75%	134	27 20.14%

With d iarrhoea Chi-square value = 3.1024 P value = 0.3761 P > 0.05

Without diarrhoea Chi-square value = 6.6289 P value = 0.08471 P > 0.05

DISCUSSION

Little is known of the prevalence of calves cryptosporidiosis in Algeria for comparison purposes. Only a few published reports of cryptosporidiosis in animals are available from Algeria.

The current study revealed that 214 fecal specimens collected from calves from birth to 60 days of age were subjected to estimate the presence of Cryptosporidium spp Staining of Cryptosporidia by modified Ziehl- Neelsen technique [25]. It is evident from these data shown that, *Cryptosporidium* spp. oocysts are present in calves from the two areas in eastern Algeria.

Cryptosporidium spp. oocysts are present in calves from the two regions in easthern Algeria. The prevalance figure (27.10%) found in the present study was comparable to anothers previous works reports in world. There were relatively higher prevalence in different countries 35.5% in USA, 33.5% in Vietnam, 27.9% in UK, 28.5% in Sirlanka and 47.9% in Spain, were reported, respectively by Santin *et al.* [26], Nguyen *et al.* [27], Brook *et al*. [28] and Castro-Hermida *et al.*[29]. On the other hand, lower prevalence were reported of 17.9% in France, 17.6% in central Ethiopia, 19.2% in Zambia and 11.9 in USA [30-33]. These differents in prevalence rates may be due to geographical and environmental differences, level of care and hygiene in the farm, husbandry system of livestock production system and susceptibility animals that related to age difference. Besides the sensitivity of the diagnostic methods utilized might also the cause of this difference [34, 35].

The results of present study also revealed that Cryptosporidium spp is equally pathogenic for both calves in Annaba (25.39%) and calves in Eltarf (29.50%) in all age groups. The prevalence difference observed in the two areas was not statistically significant.(P value = 0.8792P P > 0.05).

The results in this study revealed that both the sexes are equally susceptible, which is congruent with [36].Our finding showed no significant difference (P>0.05) between the rate of infection between female and male calves as indicated by others [37,38].The reason for the current observation is however not obvious.Most researchers believe that there is no correlation between the prevalence of *Cryptosporidium* spp and the animals sex.

Our finding in this study, that *Cryptosporidium* infection was detected in 18 (56,25%), 32 (24,24%), 8 (16%) of calves in the age groups; 1-15, 16-30 and 31-60 days old, respectivly. The infection rate was significantly decreased by advancement of age and data from the

available literature concluded that the prevalence ofCryptosporidium infection in cattle reduces as the progress of the age of the animal [39-41]. Indeed, *Cryptosporidium* oocysts was most commonly detected in 1-15 days of age calves than that of the other age groups. The high prevalence of *Cryptosporidium parvum* in 4-15 days of age calves is similar to previous reports [42, 43].

Also Cryptosporidium spp. oocysts were detected in cows as young as 3 days old to adults; the prevalence was significantly higher in suckling calves [44-47]. Age appeared to be an important factor that influences the occurrence of the Cryptosporidium.The same results have been reported by [48], who have demonstrated that the infection rates of 53.8, 14 and 7.7% in calves aged <1.5 months, 1.5–4 months and 4–24 months, respectively.Mtambo *et al*.(1997) have reported that the prevalence of *Cryptosporidium* infection was higher in calves <3 months of age as compared to weaned calves and adults [49].

For instance, [50] recorded that calves under 4 months were 13 times more likely to be infected with *Cryptosporidium* than older ones. [51] have reported an infection rate of 25% in calves 8–14 days old, a rate that apparently decreased when the age of calves increased.
The occurrence of high infection rates in this category 1-15 days may be attributed to the immatured immunity system of the animal at this age. Additionally, some of the Synergic infection of enteropathogens, such as Rota virus,Corona virus, Salmonella and *E.coli* can result in theimmunecompromised condition and the newborn animal will be more susceptible to the *Cryptosporidium* infection [52].

In another study, infection was observed in 18% of calves less than one month in Shahre-kord [53]. The infection rate was reported 4.1% by Yakhchali and Gholami (2008) that the maximum rate was in 1-4 months calves [54], and this finding is similary of our findings that the maximum rate of infection was reported in calves with fewer than 60 days old.

In current study, age of studied calves was one of important factors in prevalence of the infection and the maximum of the infection rate was observed in infant calves with less than 2 months age [55]. Therefore, it appears that age of calves is a potential risk factor for the infection and cryptosporidiosis was seen in animal with lower age. Thus, there was a significant association between Cryptosporidium infection and age (Chi-square value=8.7008 P=0.0335 P<0.05).

CONCLUSION

In this study area, the prevalence in clinical and assymptomatic cryptosporidiosis cases was found as 27.10% in aestern Algeria and the area is contaminated with Cryptosporidium sp. Therefore they represent an important starting point for further studies and needed to make moleculer studies to specify the Cryptosporidium sp and to identify agent genotypes withe genetic characterization of the isolates as the source of contaminations and zoonotic potentiel (Moleculer Epidemiolgy). Moreover, studies to understand the dynamics of transmission cycles and the genetic diversity of Cryptosporidium spp. on the farms and to identify if possible alter management practices that are risk factors for human infections, should be initiated and undertaken.

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