Prevalence and associated risk factors of cryptosporidiosis among cattle in the region of eastern Algeria

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Abstract

A cross-sectional study was undertaken to determine the prevalence and assess the potential risk factors for *Cryptosporidium* spp infection among the calves in and around Annaba town, eastern Algeria in both traditional breeding farms and smallholders, and in cross and local calves breed.

Three hundred and forty fecal samples were collected from the calves younger than four months, and screened for *Cryptosporidium* spp oocysts by microscopy.

The overall prevalence rate was 24.11%. Associations between each independent variable including age, type of rearing room, hygienic condition of the calves and cryptosporidiosis infection were statistically significant ($p<0.05$) in both univariate analysis and logistic regression. The cryptosporidiosis prevalence was significantly higher in diarrheic feces calves (30.20 %) comparing to the calves without diarrhea (16.21 %).

Coprological evidence of *Cryptosporidium* spp. was revealed in the calves with associated risk factors such as diarrhea and hygienic conditions. A further molecular investigation, monitoring, and reporting of outbreaks in the region are necessary to devise control strategies and limit transmission to humans.

Keywords: Algeria, calves, *Cryptosporidium* spp, prevalence, risk factors

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Introduction

Cryptosporidiosis is a parasitic protozoan disease defined as a global parasitic zoonosis caused by parasites belonging to the genus *Cryptosporidium* that infect the gastrointestinal epithelium of a wide range of vertebrates (Rodrigues et al., 2016). Among domesticated animals, bovines are the most studied and most frequently affected group, especially the young (Aquino et al., 2015), where *C. parvum, C. bovis, C. ryanae* and *C. andersoni* are distributed depending on age and species (Santín et al., 2004).

Cryptosporidium parvum, infecting human and most animal species, is considered the major contributor to zoonotic transmission of cryptosporidiosis (Robertson et al., 2014) with the morbidity rate varying from 5 to 11%. This is considerably high value when associated with other infectious agents, weakness due to nutritional status and immunosuppression of the calves (Couto and Bomfim, 2012). Farm animals are usually susceptible to several species of *Cryptosporidium* responsible for the aqueous diarrhea syndrome as well as the abdominal pain, dehydration, weight loss, growth delay and, in many cases, animal decease (Galvao et al., 2012). It is considered as producing a high-risk infection for newborn and immunosuppressed individuals, thus causing significant economic losses, either directly due to retarded growth, or due to even mortality in neonates/juveniles and decreased milk production in adults (Torsein et al., 2011). It has also been recognized as a principal reason of wet diarrhea in early weaned ruminants, causing high morbidity and fatality (Cho et al., 2013). The high prevalence and great oocysts shedding among the calves play an important role as a direct source of infection, with the oocysts persisting for long periods in the suitable environment (Castro-Hermida et al., 2002). The fecal-oral route remains the main transmission path being linked to water and food intake as well as contact with infected animals and/or humans (Smith et al., 2015).

Most *Cryptosporidium* species from birds and mammals are considered responsible for zoonosis by contact or ingestion (Galvao et al., 2012). Danisova et al. (2017) demonstrated that rodents could play an important role in transmitting infection due to zoonotic species (*C. parvum*, *C. hominis*, *C. suis* and C. *scrofarum*). Recently, cryptosporidiosis has also been recognized as the second cause of various types of diarrhea in children in developing countries (Platts-Mills et al., 2015).

In Algeria, only few research works about cryptosporidiosis have been published, and the first documented report on *Cryptosporidium* infection in Algeria reported a prevalence of 16.97% in calves (Khelef et al., 2007). Moreover, available information concerning this disease including prevalence, distribution and risk factors are unknown or inadequate.

This study is aimed at assessing the prevalence of *Cryptosporidium* spp. infection in calves in two different breeding systems, and studying the influence of the related risk factors on the prevalence of this infection in and around Annaba town, eastern Algeria.

Material and Methods

Study Population

Traditional breeding and smallholders' farms were sampled, and the feces were examined for *Cryptosporidium* spp. The sampled animals (males and females) were categorized into four groups as: group I = 0-30 days old, group II = 31-60 days old, group III= 61-90 days and group four = 91-120 days.

Study design and sampling strategy

A cross-sectional study was undertaken from October 2016 to May 2017 to determine the prevalence of cryptosporidiosis in calves, and evaluate associated risk factors in both traditional breeding farms and smallholders', and in cross and local calves breed. Information on the farms' structure and calves' management were registered. Information obtained through the owner/responsible interviews and the observations made during the visit were collected on the questionnaires.

Answers to these questions were used as variables to explain and analyze the results for *Cryptosporidium* spp. Hygienic conditions on the farms were classified according to the following criteria: good (good sanitation); medium (satisfactory sanitation); and poor (unsatisfactory sanitation) based on the aspects such as accumulation of feces, odor, waste drainage, cleanness of the floors and animals, barn ventilation and light source, and animal stocking. Type of the floor in the calf houses (noncemented, partially cemented) as well as the type of rearing room were subjectively assessed. Age and sex were also recorded.. At the same time, information on diarrhea and other ill health signs in calves were collected. Physical condition estimation (related animal body gain and ill health signs) was another factor recorded during the sample collection.

Fecal sample collection

During the visit to each farm, a single fresh rectal fecal sample was taken directly from the rectum using sterile plastic gloves, and placed in technically sterile, suitable leak-proof plastic containers, tightly closed and labeled. The samples were transferred into the stool containers, preserved in 10% formalin, and quickly transported after collection.They were further stored at 4˚C and processed within 48 h (Parasitological Laboratory Ibn Sina, Hospital of Annaba). At the time of sampling, the name of the farm (owner), date of sampling, consistency type of feces (score 0-1, 0: Non-Diarrheic, 1: Diarrheic) and the age, sex, breed and address were recorded for each calf on a data recording format.

Parasitological investigation

The samples were analyzed by formol-ether concentration technique (Allen and Ridley, 1970) with the minor changes. About 1 g of stool specimen was emulsified in 4 ml of 10% formol saline. Subsequently, the suspension was filtered through gauze into a vase then 4 ml formol, and 2.5 ml of diethyl ether were added. The samples were centrifuged at 1000 g for 5 minutes. After removing the supernatant, 2 drops of the pellet were smeared onto a slide and stained following modified Ziehl-Neelsen staining (Henriksen and Pohlenz, 1981). The observation was made under the microscope (x1000). The *Cryptosporidium* spp. oocysts were visualized as densely stained red round bodies clearly distinguishable against a green background.

Data Analysis

For each individual animal examined, information relevant to epidemiological investigation were registered, entered and managed on a database established in Microsoft Excel spreadsheet for Windows 2007. Statistical analysis was performed with SPSS software version 16 for Windows. Descriptive statistics were used to estimate the prevalence of the disease across the individual calves and farms management factors. Additionally, the associations between the prevalence of cryptosporidiosis and its possible risk factors were first screened in an univariate analysis using Chi-square test. Logistic regression analysis was used to calculate odds ratios and 95% confidence interval (CI) for risk factors of *Cryptosporidium* spp infection.

Results

Out of 340 calves fecal samples examined, 24.11 % were found positive for cryptosporidiosis.

Recorded were the prevalence rates at 21.95 % and 24.41 % for the crossbreed and local breed calves, respectively, with no significant difference between these breeds (Table 1).

The husbandry practice was of 26.60 % in traditional and 19.67% in smallholders managed calves, with no significant difference .

In this study, the prevalence of cryptosporidiosis infection was higher in partially cemented (25.83%) than cemented floor facilities (13.33%), but without significant difference.

A strong association was observed between the type of rearing room and risk of *Cryptosporidium* spp infection: higher prevalence was recorded in animals reared in the closed type of housing system (without outdoor access) as compared to one with outdoor access (open type). From 340 calves examined, 7.95%, 17.33%, and 48.03

% prevalences were obtained from good, moderate and poor hygienic farm conditions , respectively. There was a statistically significant association between infection with cryptosporidiosis and the hygienic status (Table 2).

Prevalence of *Cryptosporidium spp* infection was higher (30.20%) with diarrheic fecal consistency, while the lowest (16.41%) was recorded with normal fecal consistency. The prevalence of infection peaked in the calves aged 1-30 days in both diarrheic and non-diarrheic groups. The lowest prevalence was observed in calves of 91- 120 daysold group. There was a significant difference between the prevalence in calves with Cryptosporidiosis and fecal consistency (Table 2). The odds ratios for risk factors (final model) are shown in Table 4.

Table 1: Univariate analysis and relationship between potential risk factors and cryptosporidiosis infection in terms of calf-level variables

Risk factor	# Examined calf	# Positive case	Prevalence (%)	X^2	P- value
Breed					
Cross	41	9	21.95	0.1196	0.729
Local	299	73	24.41		
Sex					
Female	186	45	24.19	0.001	0.971
Male	154	37	24.02		
Age (days)					
0-30	94	40	42.55	30.346	<0.00001
31-60	90	23	25.55		
61-90	81	12	14.81		
91-120	75	7	9.33		
Physical condition					
Healthy	147	44	29.93	1.146	0.564
Weak	169	34	20.11		
Emaciated	24	4	16.66		
Total	340	82	24.11		

Table 2: Relationship between potential risk factors and cryptosporidiosis infection

Risk factor	# Examined calf	# Positive case	Prevalence (%)	X^2	P- value
Breeding type					
Traditional	218	58	26.60		
Small holders	122	24	19.67	2.054	0.152
Floor condition					
Partially cemented	295	76	25.83	3.296	0.069
Cemented	45	6	13.33		
Type of rearing room					
Without outdoor access	150	48	32		
Without door access	190	34	17.89	11774	0.0006
Hygienic conditions					
Good	88	7	7.95		
Medium	150	26	17.33 %	48.228	<0.00001
Poor	102	49	48.03		
Total	340	82	24.11		

Table 3: Prevalence of *Cryptosporidium* spp with age group and diarrheal status of calves

Age (days)	No examined	positive(%)	Status of calves			
			Diarrheic		Non-Diarrheic	
			No examined	positive(%)	No examined	positive (%)
0-30	94	40 (42.55)	61	30 (49.18)	33	10 (30.30)
31-60	90	23 (25.55)	38	14 (36.84)	52	9(17.30)
61-90	81	12 (14.81)	43	8 (18.60)	38	4 (10.52)
91-120	75	7 (09.33)	50	6 (12)	25	1 (4)
0-30	94	40 (42.55)	61	30 (49.18)	33	10 (30.30)
			192	58 (30.20)	148	24 (16.21)
				$X^2 = 8.941$	P value = 0.003	

Table 4: Odds ratios (OR) of risk factors for calves and Cryptosporidiosis infection

Risk factor	OR	95%-CI	P-value
Age (days)			
0-30	1		
31-60	2.157	(1.154,4.034)	0.015
61-90	4,259	(2.039,8.899)	<0.001
91-120	7.196	(2.988,17.329)	<0.001
Type of rearing room			
Without outdoor access	1		
Without door access	2,64	(1.498,4.653)	0.0006
Hygienic conditions			
Good (good sanitation)	1		
Medium (satisfactory sanitation)	0.412	(0.171,0.994)	0.043
Poor (unsatisfactory sanitation)	0.094	(0.039,0.222)	<0.001
Diarrheal status of calves			
Diarrheic	1		
Non-Diarrheic	2.236	(1.310,3.817)	0.003

Discussion and conclusions

The overall prevalence of 24.11 % is in accordance with 26.15 % reported by Joute et al. (Joute et al., 2016).

On the other hand, the present rate is relatively higher compared to the prevalence reports from Ethiopia, Egypt, Malaysia, England and Algeria with the prevalences of 7.8 %, 13.6%, 15.9%, 11.9% and 16.97 %, respectively (Wegayehu et al., 2013; Ameret al., 2013; Nur-Hazirahet al., 2016; Khelef et al., 2007).

Our results are lower than those reported by Muhid et al. (27) and Santos et al. (28) with the infection rate of 75 % in Malaysia and 60% in Brasil, respectively.

These differences can be explained by the geographical, environmental and agro-ecological reasons , differences in levels of farms' management , husbandry system of livestock, production system and animals' susceptibility related to age (Geurden et al., 2006). Besides, the sensitivity of the diagnostic methods utilized might also cause this difference; other tests with higher sensitivities such as PCR should be employed (Santın et al., 2009).

There was no statistically significant association (P>0.05) between the breed and cryptosporidiosis infection, which is due to either equal likelihood of being infected with cryptosporidiosis or no difference in protective immunity for the disease.

Both male and female calves are equally susceptible with no statistically significant association (P>0.05) (Male: 24.02%, Female: 24.19 %), which is in agreement with the report of Venu et al. (2013). This can be explained by the equal chance of being infested by *Cryptosporidium oocysts* and by equal protective immunity.

A significant difference (P<0.05) was found between the prevalence rate of *Cryptosporidium* spp. and calve age. The infection rate was significantly decreased by the advancement of age, with the highest prevalence found in calves aged 0 to 30 days (42.55 %), and the lowest in animals aged more than 90 days (9.33 %). These findings are in support of several other findings where cryptosporidiosis was found to be associated mainly

with age (Paul et al., 2008). Likewise, a high prevalence of *Cryptosporidium* parvum infection was reported by Bhat et al. (2013) but also by Al-Robaiee (2014) in dairy calves under one-month-old with 79.41 % and 71.9%, respectively.

The occurrence of high infection rates in this age category was attributed to the poor immunity in the newborn calves (Garroet al., 2016).

In this study, the physical condition does not influence the occurrence of cryptosporidiosis infection in relation with the same chance to access oocysts.

Calves in traditional herds had a significantly higher prevalence compared to calves in the smallholder herds ($P<0.05$). This is in agreement with another study from Tanzania, reporting prevalences of 63.6 % and 36.4 %, respectively, in the smallholder and traditional production systems (Swai and Schoonman, 2010). This finding demonstrated the disease being important in both breeding systems.

The higher prevalence observed in calves kept at partially cemented floor is most likely directly related to cleaning of cement compared with other types of flooring (sand, earth or gravel) that tend to accumulate humidity and excrement, conjugated to longer periods between cleanings so oocysts survive for long, and calves or their feed have contact with that material. There is not a statistically significant difference ($P > 0.05$) between floor condition groups, so our finding disagrees with other reports (Mohammed et al., 1999; García-Romo et al., 2014).

In the present study, there is a significant association between a type of the rearing room and the rate of prevalence of infection ($p<0.05$) in that the prevalence of *Cryptosporidium spp* infection in confined dairy calves showed a high infection rate than the calves with outdoor access. O'Handley (2007) reported that *Cryptosporidium parvum* was highly prevalent in young dairy calves and confined beef calves, and occurs rarely in free range and adult cattle. This observation is corroborated by the report by Alemayehu et al. (2013). This could be attributed to the fact that probability of infection may increase in animals confined in a small area and in housed animals, and the surrounding environment may be conducive to infection.

Calves raised in farms with poor hygiene showed higher prevalence (48.03 %, $p<0.05$) than those in farms with relatively better hygiene (7.95 %), mainly due to poor hygiene in the calving and calf housing areas but also poor management of housing. In another hand, the presence and accumulation of discarded fecal matter, as observed in our study, represent a source of contamination with infective oocyst. Other studies have also reported infection correlated with hygienic defections (Mohammed et al., 1999; García- Romo et al., 2014).

Our finding that diarrhea represents a higher risk for this disease is in accordance with other authors (Lin et al., 2012; Aguirre et al., 2014).

One reason that can explain this fact is that diarrhea provides a better chance for oocyst excretion and dissemination in comparison with non-diarrhea.

However, other study has reported that infection is not associated with the occurrence of diarrhea (Nur- Hazirah et al., 2016), meaning that several risk factors are involved. The excretion of oocysts by asymptomatic calves reveals a transporter status and may act as a reservoir for transmission to susceptible calves.

The overall prevalence of *Cryptosporidium* spp was high at 24.11 %. Furthermore, it was observed that age, diarrhea, type of rearing room and hygienic condition are the potential risk factors.

Further molecular epidemiology investigations are required to determine the *Cryptosporidium* species and genotypes in Algerian cattle, and to establish a true picture of the infection in the region, which is necessary to devise control strategies and limit transmission to humans.

Competing Interests

The authors declare that they have no competing interests.

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Prevalencija i riziko faktori kriptosporidioze stoke u regiji istočnog Alžira

Apstrakt

Provedena je studija u svrhu određivanja prevalencije i procjene potencijalnih riziko faktora infekcija teladi izazvanih sa *Cryptosporidium* spp u gradu Annaba i njegovoj okolici, u istočnom Alžiru. Istraživanje je provedeno na tradicionalnim farmama za uzgoj teladi, i kod malih uzgajivača, na križancima i domaćim vrstama.

Prikupljeno je tristo četrdeset uzoraka fecesa teladi starosti do četiri mjeseca, nakon čega je mikroskopski izvršeno ispitivanje na oociste *Cryptosporidium* spp.

Ukupna prevalencija iznosi 24.11%. Testovima univarijantne analize i logističke regresije je dokazana statistički signifikantna povezanost ($p<0.05$) između pojedinih neovisnih varijabli, uključujući starost teladi, vrstu i higijenske uvjete smještaja.

Prevalencija kriptosporidioze je signifikantno veća kod teladi sa dijarejom (30.20 %) u odnosu na telad bez dijareje (16.21 %).

Neophodna su dalja molekularna istraživanja, monitoring i izvještavanje o epidemijama bolesti u regiji kako bi se kreirale strategije kontrole i ograničio prenos bolesti na ljude.

Ključne riječi: Alžir, telad, *Cryptosporidium* spp, prevalencija, riziko faktori