RESEARCH ARTICLE

STUDY OF THE PREVALENCE OF *CRYPTOSPORIDIUM* SPP. IN CATTLE FARMS IN THE LAGHOUAT REGION, SOUTHERN ALGERIA

Radhwane Saidi[1], Mourad Becheur[2], Mourad Hamiroune[3*], Khadidja Bailiche[4], Lilia Yasmine Laméche[4]

[1] Laboratory of Biological and Agronomic Sciences, Department of Agronomic Sciences, Faculty of Sciences, University of Laghouat, Route de Ghardaia, Laghouat 03000, Algeria

[2] Department of Agronomic Sciences, Faculty of Nature and Life Sciences, University of Laghouat, Route de Ghardaia, Laghouat 03000, Algeria

[3] Laboratory of Exploration and Valorization of Steppe Ecosystems, Faculty of Natural and Life Sciences, University of Djelfa, PO Box 3117, Djelfa 17000, Algeria

[4] Department of Biological Sciences, Faculty of Sciences, University of Laghouat, Route de Ghardaia, Laghouat 03000, Algeria

*Corresponding author:
Dr. Mourad Hamiroune
Address: Laboratory of Exploration and Valorization of Steppe Ecosystems, Faculty of Natural and Life Sciences, University of Djelfa, PO Box 3117, Djelfa 17000, Algeria
Phone: +213 27900302
ORCID: 0000-0003-0704-595X
E-mail: m.hamiroune@univ-djelfa.dz

ABSTRACT

The objective of the present work was to detect *Cryptosporidium* spp. infection in cattle farms in Laghouat, Algeria. Our study was carried out for 3 months (February, March and April 2018) and on 300 faecal samples taken from 300 cattle. For this purpose, we used the modified Ziehl–Neelsen staining technique to identify cryptosporidium spp. The parasite prevalence rate was 43.5%. The statistical analysis of the influence of certain variation factors (sex, age, type of breeding, presence of diarrhea and breed) on the prevalence of *Cryptosporidium* spp. revealed no significant effect ($p > 0.05$) for the breed, sex, type of breeding and deworming. However, the effect of age was very significant ($p < 0.01$) and that of the presence of diarrhea was also significant ($p < 0.05$). These results reflect the risk posed by *Cryptosporidium* spp. to cattle and to public health. This requires the implementation of a disease control and awareness program to avoid various accidents and significant economic losses. In addition, early diagnosis of the disease helps preserve animal and human health and the national economy, subsequently.

Keywords: Bovine, Algeria, *Cryptosporidium* spp., prevalence

INTRODUCTION

Cattle breeding represents one of the most important agricultural activities in the world. It plays a fundamental role at the economic, ecological, environmental and cultural levels (Faye, 2001). In Algeria, like all Maghreb countries, cattle breeding is among the oldest activities; it plays a relatively important role both in the national agricultural economy and for breeders, thus providing a considerable financial reserve (Benghida, 2021).

However, this breeding is subject to nutritional, environmental or pathological risks. Among the pathologies which can cause mortality

and/or poor zootechnical performance, we can cite parasitic diseases, including cryptosporidiosis.

Cryptosporidiosis is of considerable importance in newborn ruminants, in which it is characterized by mild to severe diarrhea, lethargy and poor growth rate (Paraud et al., 2009). The source of cryptosporidian infection is oocysts that are fully spore-forming and infectious when shed in the feces. Transmission can occur directly from calf to calf, indirectly through passive or human transmission, through environmental contamination, or through fecal contamination of food or water supplies. Diagnosis of cryptosporidiosis is based on detection of oocysts by examination of fecal smears with Ziehl-Neelsen stains, fecal flotation techniques, ELISA, fluorescently labeled antibodies, rapid immunochromatographic testing, and PCR (Witola, 2021).

Cryptosporidiosis is an emerging opportunistic parasitic disease (Chermette et Boufassa-Ouzrout, 1988). Most often asymptomatic, it can sometimes manifest clinically as digestive disorders in immunocompromised subjects in the presence of intercurrent conditions. The causative agent is an intracellular protozoan of the coccidia family belonging to the genus *Cryptospridium* (Fayer and Santin, 2009; Squire et al., 2013). Several species have been isolated from a large number of vertebrates, including humans, in whom the

interest in this parasite is directly linked to the occurrence of epidemics since 1980.

Cryptosporidiosis has been described in many animal species, both domestic and wild. In ruminants, more precisely the bovine species, it is generally the youngest animals which are the most receptive and most sensitive to infection, while infected adult animals are few in number and asymptomatic (Fayer and Santín, 2009; Paraud et al., 2009; Silverlå at al., 2010). Currently, cryptosporidiosis is one of the leading causes of diarrheal enteritis in newborn calves (Silverlås et al., 2010). This infection causes significant economic losses in newborn ruminants due to the mortality and morbidity it causes. The average prevalence of this infection varies widely between countries and different ruminant host species (Silverlås et al., 2010).

To our knowledge, there have been only two studies on cryptosporidiosis in cattle in the Laghouat region (Chikhaoui and Touhami, 2015). Also, the national bibliography has been enriched in recent years with some research work on bovine cryptosporidiosis, particularly in some regions of the East and central North (Khelef et al., 2007; Ouchene et al., 2014). Therefore, the present study aims to evaluate the prevalence of *Cryptosporidium* spp. and risk factors implicated on cryptosporidian infection in cattle in the wilaya of Laghouat.
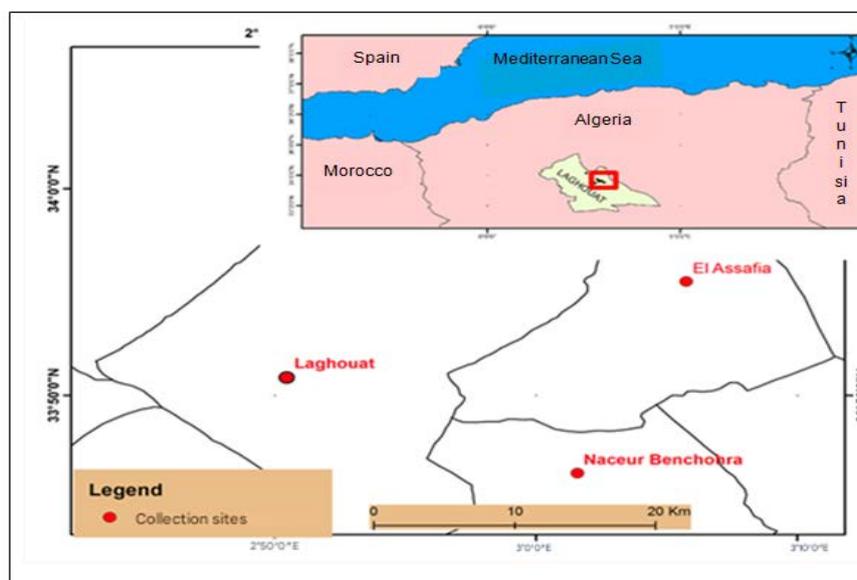

Figure 1 Location map of sampling sites (ARCGIS V10.2 software)

MATERIAL AND METHODS

Location and period of study

This study was carried out at three sites in the Laghouat region in the south of Algeria: Laghouat-City center, El-Assafia, and Bennaceur Benchohra (Figure 1). Our study was carried out for 3 months, from February to April 2018.

The coprological analyzes took place in Parasitology Laboratory of the Biology Department of Amar Telidji University of Laghouat and Laghouat Regional Veterinary Laboratory.

Animal material

The study was carried out on 13 farms, including 4 large livestock farms and 9 small dairy cow farms. A total of 300 cattle were sampled in this study. The characteristics of the animals studied are presented in Table 1.

Table 1 Characteristics of the cattle studied

Characteristics		Number of animals per study site (%)			
		Municipality of Laghouat	Municipality of Bennaceur Benchohra	Municipality of El-Assafia	Total
Gender	Female	141(47%)	35(11.7%)	20(6.7%)	196(65.3%)
	Male	76(25.3%)	13(4.3%)	15(5%)	104(34.7%)
Race	Prim'Holstein	160(53.3%)	26(8.7%)	19(6.3%)	205(68.3%)
	Montbéliarde	57(19%)	22(7.3%)	16(5.3%)	95(31.7%)
Age	From 14 days to 30 days	14(4.7%)	0(0%)	6(2%)	20(6.7%)
	From 1 month to 3 months	53(17.7%)	17(5.7%)	7(2.3%)	77(25.7%)
	From 4 months to 12 months	43(14.3%)	1(0.3%)	1(0.3%)	45(15%)
	From 1 year to 2 years	43(14.3%)	7(2.3%)	12(4%)	62(20.7%)
	More than 2 years	64(21.3%)	23(7.7%)	9(3%)	96(32%)

The characteristics of the farms visited are presented in Table 2.

Table 2 Characteristics of the farms visited during this study

Characteristics		Number of animals per study site (%)			
		Municipality of Laghouat	Municipality of Bennaceur Benchohra	Municipality of El-Assafia	Total
Type of farming	**Intensive**	127 (42.3%)	31 (10.3%)	0 (0%)	158 (52.7%)
	Semi-intensive	90 (3%)	17 (5.7%)	35 (11.7%)	142 (47.3%)
Deworming	**Dewormed animals**	105 (35%)	31 (10.3%)	15 (5%)	151 (50.3%)
	Animals not dewormed	112 (37%)	17 (5.7%)	20 (6.7%)	149 (49.7%)

Parasitology

Sampling and laboratory examination

Sampling was carried out randomly, depending on the availability and cooperation of the breeders contacted. The samples were based on animal feces, taken directly from the rectum. After collecting the feces, they were placed in sterile collection boxes, labeled (the date of collection, age, sex and possibly the breed of the animal are mentioned) then transported in a cooler to the laboratory. Samples that are not analyzed the same day are stored cold at 4°C.

In the laboratory, observation of stools with the naked eye was carried out, in order to assess in particular the color, the appearance of stools, the presence of blood, pus or mucus and observation of different forms of parasites (eggs, larvae, worms etc.). Second, to test for *Cryptosporidium* spp. oocysts, a smear was prepared for each sample. Then, the smear was colored using the Ziehl-Neelsen staining technique modified by Henriksen and Pohlenz (1981), starting by lethally spreading a drop of stool as finely as possible on a slide which will be fixed in ethanol at 95% for 5 minutes. The blade was then flamed with a Bunsen burner and covered while still hot with Ziehl fuchsin and left to act for 5 min. Then, it was rinsed with tap water until excess fuchsin was eliminated, then sprayed with one or two jets of 3% HCL in 95% ethanol,

rinsing with water each time. After rinsing with water, the slide was soaked in 0.25% malachite green or methylene blue for 30 seconds. Finally, after drying, an optical microscope observation (×100 objective) was carried out without covering a coverslip (using an immersion oil). When positive, the oocysts are colored red or pink on a green or blue background.

Calculation of total prevalence

It is the percentage ratio P (%) of the number of hosts infested by a given species of HP parasite to the total number of hosts examined HE (Margolis et al., 1982).

P (%) = HP/HE x 100

Statistical processing of data

The recorded results were grouped in an Excel 2007 file for creating graphs and calculating prevalences. The effect of the variation factors was analyzed using SPSS software (Version 20) using the Chi-square test. The difference is considered significant at a threshold of $p < 0.05$.

RESULTS

***Cryptosporidium* spp. observed in the animals studied**

Oocysts colored bright red or pink on a green background. Some may appear as empty discs,

Figure 2 Oocysts of *Cryptosporidium* spp. Observed under the optical microscope: G x 1000, immersion oil (modified Ziehl-Neelsen coloring)

while others may contain crescent-shaped elements that are characteristic of sporozoites (Figure 2).

General prevalence of *Cryptosporidium* spp.

Of the 300 cattle sampled, 164 were infected with *Cryptosporidium* spp., representing an overall prevalence rate of 45.3%

Study of the influence of certain parameters on the rate of infection by *Cryptosporidium* spp.

Influence of age

The rate of parasitism according to age shows that the infection rate of animals from 14 days to 30 days (100%) was higher than that of the other groups. Statistical analysis revealed that the difference was very significant ($p<0.001$), Table 3.

Table 3 Representation of the parasitism rate among age groups

	Number of positive cases/total number	Prevalence of *Cryptosporidia* (%)
G1 : From 14 days to 30 days	20/20	100
G2 : From 1 month to 3 months	63/77	81.8
G3 : From 4 months to 12 months	21/45	46.7
G4 : From 1 year to 2 years	24/62	38.7
G5 : More than 2 years	8/96	8.3

Influence of gender

Generally speaking, the rate of parasitism in females (47.4%) was higher than that in males (41.3%) (Table 4). However, statistical analysis revealed that the difference was not significant.

Table 4 Representation of the parasitism rate according to sex, breed, type of breeding and antiparasitic treatment

Characteristics		Number of positive cases/ total number	Prevalence of *Cryptosporidia* (%)
Gender	**Female**	93/196	47.4
	Male	43/104	41.3
Race	**Prim'Holstein**	102/205	49.8
	Montbéliarde	34/95	35.8
Type of farming	**Intensive**	74/158	46.8
	Semi-intensive	62/142	43.7
Deworming	**Dewormed animals**	69/151	45.7
	Animals not dewormed	67/149	45

Influence of race

The rate of parasitism depending on the breed, as illustrated in Table 4, shows that the infection rate in cattle of the Prim'Holstein breed (49.8%) was higher than that of the Montbéliarde breed (35.8%). However, statistical analysis did not reveal a statistically significant difference (p>0.05).

Influence of the type of breeding

The rate of parasitism depending on the type of breeding, as illustrated in Table 4, shows that the infection rate in intensive breeding (46.8%) was higher than that of semi-intensive breeding (43.7%). However, statistical analysis revealed that the difference was not significant (p>0.05).

Influence of deworming

The rate of parasitism as a function of deworming, as illustrated in Table 4, shows that there is no big difference between the infection rate in dewormed cattle (45.7%) and non-dewormed cattle (45.0%). In this sense, the statistical analysis revealed that the difference was not significant

Influence of diarrhea

The rate of parasitism depending on the presence of diarrhea or not, as illustrated in Figure 3, shows
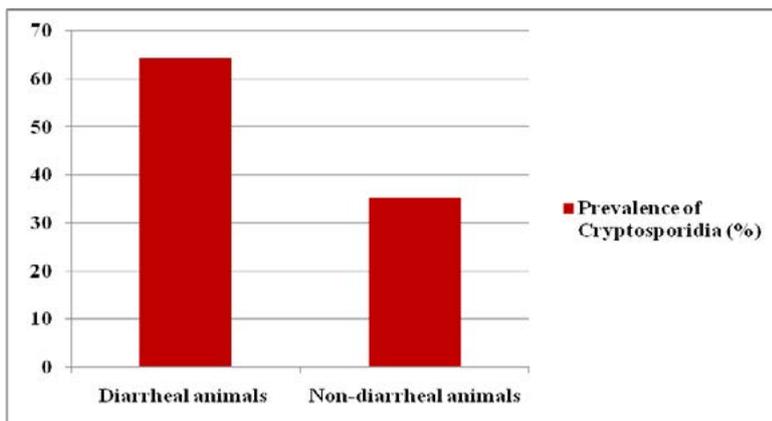

Figure 3 Graphical representation of the parasitism rate according to the presence of diarrhea

that the infection rate of diarrheal animals (64.4%) was higher than that of non-diarrheal animals (35.2%). In addition, the statistical analysis revealed that the difference is very significant (p < 0.001).

DISCUSSION AND CONCLUSION

Our results revealed a high presence of *Cryptosporidium*-infection in cattle in the studied region. Indeed, the presence of the parasite is noted in all the sites studied but in varying proportions.

The stool examination revealed a rate of 45.3% for *Cryptosporidium* spp. This value is close to that recorded by Chikhaoui and Touhami (2015) (45%) in the same region, therefore under the same climatic conditions. This last result is explained by its authors by the fact that the animal population studied was essentially composed of adults, knowing that excretion in young animals is significantly higher than in adults (Khelef et al, 2007). Our result is slightly higher than that found by Khelef et al., (2007) in eastern and central Algeria (17%). However, it is lower than that recorded by Ouchane et al. (2014) in Sétif (69.2%).

The difference in the prevalence of *Cryptosporidium* spp. recorded during our survey compared to the results of other studies must be related to the breeding management practiced, the choice of the population studied, and the climatic factors which condition the epidemiology of the parasite, such as temperature, humidity and oxygenation.

The evolution of the incidence of cryptosporidiosis over time showed that parasitic infections were higher in spring and winter during our study period (February-March-April). This could be linked less to the season factor, but to the greater concentration

of calvings over time, which would be responsible for massive contamination of calves via oocysts pre-existing in the boxes (or premises) and those newly released by young and /or adults ; this would also be linked to the great promiscuity that results. Similar observations are reported by Henriksen and Krogh (1985), Ongerth and Stibb (1989) and Dãrãbuş et al., (2001). Indeed, according to these authors, the high incidence recorded in winter is mainly attributed to the mode of animal breeding, a period during which animals are grouped in stables, which increases the spread of the parasite between conspecifics.

All of our results suggest the presence of particular receptivity of the newborn calf (from 14 to 30 days) to *Cryptosporidium* spp. in the first weeks of age (100%), with a highly significant difference (p<0.001). This is consistent with the observations of numerous authors (Stein, 1982; Sobeih et al., 1987; Ongerth and Stibb, 1989) who report the great receptivity of calves belonging to this age group to cryptosporidia, with, however, a maximum incidence in the second week after birth. Some authors report this receptivity of young people to their deficient immune state in the first days. Colostrum intake at this level plays an important role, if only in the clinical expression of diarrhea. At the end of the first week, and throughout the second and third, is the period of maximum excretion. It explains the strong positivity during this period, which is consistent with several works in the literature (Naciri et al., 1999).

It is, therefore, established that cryptosporidia mainly affects lambs less than one month old (100%) and, to a lesser degree, those aged between 4 and 12 months (46.7%).

However, 32/300 cases of infection are noted in adults. This sensitivity of adults seems to be linked to the stress of peripartum (gestation, lactation and drying off) and calving which affects certain cows, poor hygiene conditions would also be responsible (Villacorta et al, 1991). The low excretion of the parasite by adults over time is also reported in the work of Henriksen and Krogh (1985).

The relationship between the sex of the cattle studied with the rate of parasitism shows that the infection of females in general was not significantly higher (47.4%) compared to males (41.3%). We did not find an explanation for this observation in the literature available to us. The distribution of results according to the clinical status (presence or absence of diarrhea) of cattle is consistent with what is reported by other researchers: cryptosporidia are more isolated in diarrheal subjects (64.4%) compared to those without diarrhea (35.2%). Indeed, among the former, depending on the studies, the positivity rate varies from 10 to 76% (Heine and Boch, 1981; Ongerth and Stibbs, 1989), while among the latter the carriage frequency varies from 4% to 37%. (Nagy et al., 1980; Pivont et al., 1981; Heine and Boch, 1981).

Some authors have shown a significant correlation between the presence of diarrhea and the excretion of *Cryptosporidium* (Naciri et al., 1999; Castro-Hermida et al.,2002).

On the other hand, according to the study by Atwill's team dating from 1999, there is a weak correlation between the presence of liquid faeces and the excretion of the protozoan. In general, the presence of blood and liquid faeces is rather associated with a coexistence between *C. parvum* and other enteropathogenic agents (mixed infection with rotavirus, coronavirus, *Salmonella*, or others) (Naciri et al., 1999).

Regarding the influence of breed on cryptosporidian prevalence, 49.8% of Prim'Holstein breed (black magpie) cattle were infested with *Cryptosporidium* spp. compared to 35.8% in the Montbéliarde breed (red magpie). However, the difference was not significant. The black piebald breed is much more dairy than meat; on the other hand, the red magpie is mixed with a meaty tendency. The absence of the effect of this factor would be explained by the fact that the breeds studied were both improved.

Other authors have also studied the breed effect (Chikhaoui and Touhami, 2015) by comparing receptivity to *Cryptosporidium* spp. between local breed cattle and those of cross breeds. Statistical analysis revealed that the difference was not significant (p > 0.05).

The present study revealed no effect of the breeding method on the infection rate of *Cryptosporidium* spp. Indeed, cattle raised in intensive mode were infested in an almost similar manner to those raised in semi-intensive mode. It also diverges with the results of Ghesquier et al. (2003) and Marechal (2004) who confirm that the high concentration of livestock in livestock premises constitutes a contributing cause of the inter-individual spread of this parasite.

The administration of antiparasitic treatment as a preventive measure did not have a significant effect on cryptosporidian prevalence between treated and untreated animals. This confirms the literature data which note the ineffectiveness of various molecules tested to fight against this parasite. Indeed, *Cryptosporidium* holds a unique position in the host cell since the parasite is intracellular but extra-cytoplasmic (Manent-Manent, 2014). It thus escapes the intracellular action of antiparasitic drugs. In addition, controlling environmental contamination is difficult due to the very high resistance of oocysts in a natural environment but also in the presence of common disinfectants (Manent-Manent, 2014).

The results of this study confirm that the prevalence of bovine cryptosporidiosis varies from one animal to another. Infection with this disease is considered an important indicator of insufficient control of *Cryptospridium* spp. which takes animals as its main reservoir, which constitutes a risk for animal and human health in the event of contact or consumption of the oocyst of this parasite. This requires increasing the number of cattle checked each year in order to preserve animal health and consumer health, which is directly linked to the eradication of bovine cryptosporidiosis through medical and sanitary interventions.

For this reason, important are awareness and popularization among breeders, consumers and other stakeholders in the veterinary and public health sector. In addition, it would be very useful to carry out systematic screening on all farms in the country through general and specific laboratory examinations for confirmation. It is obligatory for cattle farms where the breeder has an agreement with the Directorate of Veterinary Services (DVS) for the carrying out of early detection tests for the disease, which will help to better fight against the disease in cattle and preserve the health of consumers and thus, ultimately, bring benefits to the entire economy of the country.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTIONS

Conception: RS; Design: RS, MH; Supervision: RS, MB, MH; Materials: KB, LYM; Data Collection and/or Processing: KB, LYM; Analysis and/or Interpretation: RS, MB, MH; Literature Search: MH; Writing – Original Draft: RS, MB, MH; Critical Review: RS, MH

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STUDIJA PREVALENCE *CRYPTOSPORIDIUM SPP* NA FARMAMA ZA UZGOJ STOKE U REGIJI LAGHOUAT U ALŽIRU

SAŽETAK

Cilj našeg istraživanja je otkrivanje infekcije sa *Cryptosporidium* spp. na farmama stoke u Laghouatu u Alžiru. Istraživanje smo provodili 3 mjeseca (februar, mart i april 2018. godine) na 300 fekalnih uzoraka prikupljenih od 300 goveda. U tu svrhu smo koristili modificiranu tehniku bojenja po Ziehl–Neelsenu sa ciljem identifikacije Cryptosporidium spp. Prevalenca parazita je iznosila 43.5%. Statistička analiza utjecaja pojedinih varijacijskih faktora (spol, starost, način uzgoja, prisustvo dijareje i pasmina) na prevalencu *Cryptosporidium* spp. nije otkrila statistički signifikantan utjecaj ($p>0.05$) pasmine, spola, načina uzgoja i deparazitizacije. Međutim, utjecaj starosti je bio statistički veoma signifikantan ($p<0.01$), kao i prisustvo dijareje ($p<0.05$). Ovi rezultati odražavaju rizik koji *Cryptosporidium* spp. predstavlja kako za stoku tako i za javno zdravstvo. Ovo zahtjeva implementaciju kontrole bolesti i program podizanja svijesti sa ciljem izbjegavanja nesreća i signifikantnih ekonomskih gubitaka. Osim toga, rano dijagnosticiranje bolesti pomaže očuvanju zdravlja životinja i ljudi, a posljedično i ekonomije.

Ključne riječi: Alžir, bovini, *Cryptosporidium* spp, prevalenca