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First report on *Cryptosporidium parvum*, *Escherichia coli* K99, rotavirus and coronavirus in neonatal lambs from north-center region, Algeria

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ABSTRACT

The etiology of neonatal diarrhea is multifactorial and remains one of the greatest health problems in sheep livestock farming. Faecal samples from 559 neonatal lambs aged less than 30 days from 30 sheepfolds located in the north-center region of Algeria were screened with pathogen-specific antigen ELISA for *Cryptosporidium parvum*, *Escherichia coli* K99, rotavirus, and coronavirus. Of the 559 lambs, 312 (58.81 %), 155 (27.72 %), 72 (12.88 %) and 20 (3.57 %) were positives for *C. parvum*, *E. coli* K99, rotavirus and coronavirus antigens, respectively. The prevalence of *C. parvum* was the highest ($p < 0.0001$). *C. parvum*, *E. coli* K99, rotavirus and coronavirus were observed in 23 (76.66 %), 17 (56.66 %), 9 (30 %) and 3 (10 %) sheepfolds, respectively. Compared to age, the prevalence of *C. parvum* was highest during the second and third week of age ($p < 0.001$). In contrast, other pathogens were found to be more frequent in lambs aged ≤ 7 days ($p < 0.001$). The number of lambs with diarrhea was 280 (50.09 %) of which 280 (100 %), 127 (45.35 %), 52 (18.57 %) and 10 (3.57 %) were found to be infected with *C. parvum*, *E. coli* K99, rotavirus and coronavirus, respectively ($p < 0.0001$). In various combinations, mixed infections were detected only with *C. parvum*. This is the first report of *C. parvum*, *E. coli* K99, rotavirus, and coronavirus in ≤ 30 -days old neonatal lambs in Algeria. Special attention should be given to the first colostrum feeding, hygiene of the farm, prevention and control measures for a better prevention of neonatal diarrhea in lambs.

1. Introduction

Neonatal diarrhoea remains one of the greatest health problems in ruminant livestock production, resulting in significant economic losses due to morbidity and mortality, treatment costs and reduced growth rates of affected newborns [1,2]. Its etiology is multifactorial, resulting from an interaction between the animal and its environment, nutrition and the pathogens involved [3].

The etiology of this diarrhoeal syndrome can be infectious (viruses, bacteria and protozoa) or due to non-infectious factors such as herd management, nutritional and immunological status of the host [1,4,5]. Infectious diarrhoea in lambs is most often due to different types of enterotoxigenic *Escherichia coli*, *Cryptosporidium parvum*, Rotavirus, and Coronavirus or a combination of these pathogens. They can cause clinical disease and/or may lead to suboptimal growth rate of affected animals [6–11].

However, in a neonatal diarrhoea syndrome, it is difficult to situate

the exact etiological role of each of these pathogens, due to their action which can be isolated or associated, producing practically similar clinical signs. Moreover, epidemiological studies have shown that the diversity in the degree of association between these agents is often fatal when the infections are mixed [6,7,12].

In Algeria, the overall sheep population was estimated at 28,393,602 [13]. Although, neonatal diarrhoea is one of the major preoccupations of livestock farmers in Algeria. However, to date no studies have been carried out in Algeria to investigate the main agents of neonatal diarrhoea in sheep (*Cryptosporidium parvum*, *Escherichia coli* K99, rotavirus and coronavirus). The majority of publications have only concerned cattle [14–16] and the very few studies that have been carried out on sheep are concerned only *Cryptosporidium* spp [17,18].

The present study was therefore conducted for evaluate the comparative role of *Cryptosporidium* spp and other enteric pathogens known as major agents of neonatal diarrhoea in lambs on 30 farms located in the north-central region of Algeria.

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2. Materials and methods

2.1. Study area and sample collection

The study was carried out between January 2014 and December 2016 and concerned 559 lambs aged less than 30 days. Lambs were classified into four age groups: ≤ 7 days, 8–14 days, 15–21 days, 22–30 days.

The study was carried out in 30 sheepfolds with extensive farming methods, where sheep remain on pasture for the most part of the day. The study was carried out in north-center region of Algeria: Laghouate (33°47'59" N 2°51'54" E), Djelfa (34°40'22" N 3°15'46" E), Medea (36°15'51" N 2°45'14" E) and Bouira (36°22'29" N 3°54'07" E) (Fig. 1).

Faecal samples were taken using a rectal swab from each lamb in a sterile plastic vial. The samples were then transported to the laboratory at 4 °C. Farm of origin, the date of sampling and the age of each animal were recorded. The aspect of the faeces was registered: diarrhoeal/non-diarrhoeal.

2.2. Laboratory analysis

Faecal samples were screened for antigens to *C. parvum* and the other enteric pathogens using a commercial faecal antigen ELISA test (Bio-X Easy-Digest, Bio K 151; Bio-X Diagnostics, Belgium). This kit simultaneously detects specific antigens from *C. parvum*, *E. coli* K99, rotavirus, and coronavirus and the tests were performed according to the kit manufacturers' instructions.

2.3. Statistical analysis

Detection rate of prevalence in diarrheic lambs than in no-diarrheic and in the different age groups were compared by chi-square test (χ^2) test. For multi-variable comparisons, one-way ANOVA was conducted, followed by Tukey-Kramer testing using the program R software version 3.0.1 (R Core Team 2013). Differences were considered significant at $p < 0.05$.

3. Results

Of the 559 lambs, 312 (58.81 %), 155 (27.72 %), 72 (12.88 %) and 20 (3.57 %) tested by faecal antigen ELISA were positive for *C. parvum*, *E. coli* K99, rotavirus and coronavirus antigens, respectively. The prevalence of *C. parvum* was the highest ($p < 0.0001$) (Table 1).

C. parvum, *E. coli*, rotavirus and coronavirus were observed in 23 (76.66 %), 17 (56.66 %), 9 (30 %) and 3 (10 %) sheepfolds, respectively. *C. parvum* was the most widespread ($p < 0.0001$).

Compared to age, the prevalence of *C. parvum* was highest during the second and third week of age ($p < 0.001$). In contrast, other pathogens were found to be more frequent in lambs aged ≤ 7 days ($p < 0.001$).

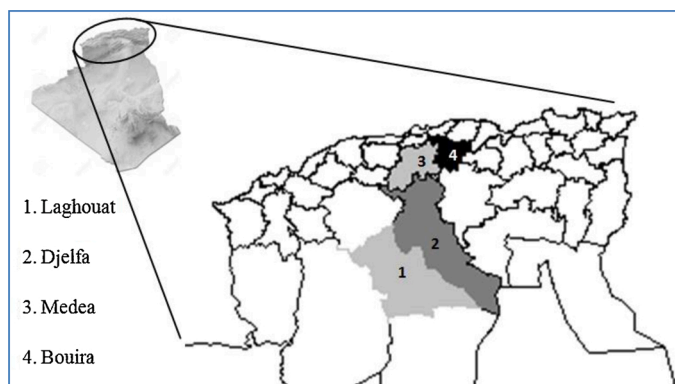


Fig. 1. Presentation of the study area.

Table 1

Prevalence of *C. parvum*, *E. coli* (K99+), rotavirus and coronavirus in the faeces of lambs.

Pathogens	N° positive samples	%
<i>C. parvum</i>	312	58.81*
<i>E. coli</i> (K99+)	155	27.72
Rotavirus	72	12.88
Coronavirus	20	3.57
Total	559	

(Table 2). Rotavirus and coronavirus were not revealed in lambs aged ≥ 15 days (Table 2).

Lambs aged ≤ 7 days were found to be more infested with *E. coli* K99 and rotavirus compared to *C. parvum* and coronavirus ($p < 0.001$) (Table 2). In the other age categories (≥ 8 days), lambs were more infested with *C. parvum* than other enteropathogens ($p < 0.001$) (Table 2).

The number of lambs with diarrhea was 280 (50.09 %) of which 280 (100 %), 127 (45.35 %), 52 (18.57 %) and 10 (3.57 %) were found to be infected with *C. parvum*, *E. coli* K99, rotavirus and coronavirus, respectively. All diarrheal lambs were found to be infected with *C. parvum*, which showed a significantly higher prevalence than other pathogens ($p < 0.0001$).

The prevalence of infection with *C. parvum*, *E. coli* and rotavirus was higher in lambs with diarrhea compared to lambs without diarrhea ($p < 0.001$). However, no significant difference was observed for coronavirus (Table 3).

C. parvum, *E. coli* K99, rotavirus and coronavirus were identified alone in 291, 146, 65 and 15 samples, respectively (Fig. 2). *C. parvum* was the most frequent ($p < 0.0001$).

The association between these pathogens was observed only with *C. parvum*, where associations of *C. parvum* with *E. coli* K99, rotavirus and coronavirus were reported in 9, 7 and 5 samples, respectively (Fig. 2).

4. Discussion

The clinical diagnosis of infectious enteritis is difficult to establish because of the frequency of clinical signs, the implication of multiple agents, and the interactions between factors that predispose the host to infection [19]. *C. parvum*, *E. coli* K99, rotavirus and coronavirus are recognized as the most important enteropathogens in diarrhea in young ruminants [20]. To our knowledge, this is the first report of neonatal lambs in Algeria in which *C. parvum* along with *E. coli* K99, rotavirus, and coronavirus infections are reported using the ELISA test for faecal antigen detection. The results showed the existence of all these pathogens with a predominance of *C. parvum*.

Prevalence of lambs' infections with *C. parvum*, *E. coli* K99, rotavirus and coronavirus revealed in this study were 58.81 %, 27.72 %, 12.88 % and 3.57 %, respectively. *C. parvum* was the most prevalent. This is in agreement with other findings ([21–24]).

The prevalence of *C. parvum* infection found in our study is consistent with the results of other studies [25–27], but was higher than 10.24 % in Iran (Mohammad et al., 2013).

C. parvum plays an important role in neonatal diarrhea in lambs [28]. In our study, a strong association between diarrhea and the presence of *C. parvum* oocysts in lamb faeces have been recorded which is in concordance with several other studies [21,23,25,29–31]. The same finding was reported also in cattle [5,32,33].

C. parvum was observed in 23 sheepfolds (67.66 %) which is comparable with Causape et al. [25], Munoz et al. [23] and higher than of [34] in Iran, Rossanigo et al. [35] in Italy and Causape et al. [25] and Pablo et al. [36] in Spain.

In this study, the higher prevalence of cryptosporidial infection was observed in lambs aged between 8–21 days. The same was reported by

Table 2
Prevalence of *C. parvum*, *E. coli* (K99+), rotavirus and coronavirus according to the age of lambs.

Age (days)	N° of samples	<i>C. parvum</i>		<i>E. coli</i> (K99+)		Rotavirus		Coronavirus	
		N° positive samples	%	N° positive samples	%	N° positive samples	%	N° positive samples	%
≤7	155	21	13.54 ^c	64	41.29 ^a	55	35.48 ^{a,b}	15	9.67 ^{a,d}
8–14	220	154	70 ^a	44	20 ^b	17	7.72 ^c	5	2.27 ^c
15–21	100	85	85 ^a	15	15 ^b	0	0	0	0
22–28	84	52	61.90 ^a	32	38.09 ^b	0	0	0	0
Total	559	312		155		72		20	

^{a,b,c,d}Values that have not the same letter in the same line are different at p < 0.05.
* These values are significantly higher than the other values in the same column at p < 0.05.

Table 3
Prevalence of *C. parvum*, *E. coli* (K99+), rotavirus and coronavirus according to diarrhoeal and non diarrhoeal faeces.

Faeces	<i>C. parvum</i>		<i>E. coli</i> (K99+)		Rotavirus		Coronavirus	
	N° positive samples	%	N° positive samples	%	N° positive samples	%	N° positive samples	%
Diarrhoeal	280	89.74*	127	81.93*	52	72.22*	10	50
Non-diarrhoeal	32	10.26	28	18.06	20	27.78	10	50

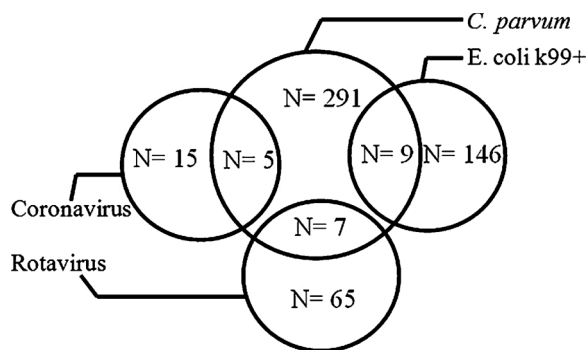


Fig. 2. Number of positive samples of *C. parvum*, *E. coli* (K99+), rotavirus and coronavirus alone or in association.

Sevinç et al. [37]. The presence of *Cryptosporidium* infection in clinically asymptomatic lambs indicated that particular age group of animals might be reservoir for the parasite [38] and the prevalence rate decreases with increase in age and, this may be due to age related immunity [39].

Neonatal colibacillosis is highly contagious in lambs [40]. Our results show that *E. coli* K99 was reported in 27.72 % of lambs which is consistent with 26 % reported in Spain [23] and higher than 19.2 %, 9.42 % reported by Fernando et al. [41] in Brazil and Malik et al. [42] in Morocco, respectively.

The most severe diarrhea was recorded in calves less than three days old with *E. coli* K99 [43]. In our study, the highest prevalence of *E. coli* K99 was observed in lambs less than 7 days old which is in agreement with Erhan et al. [44].

E. coli K99 was identified in 81.93 % of the diarrhoeal lambs in our study which is in agreement with Aklilu et al. [45] in Etiopia (84 %). *E. coli* K99 was isolated from 4% of lambs in the Netherlands [46], 0.2 % in Italy [47], 31 % in USA [48] from sheep flock and between 12.5–26.6 % [49,50] in India. These reports are much lower than our finding.

The high prevalence of *E. coli* K99 observed in our study can be attributed to delayed or insufficient in first colostrum intake, unclean shepherd and lack of implementation of appropriate prevention and control measures. Olsson et al. [51] reported that each hour of delay in colostrum ingestion during the first 12 h of age resulted in a 10 % increased risk of a lamb becoming sick. The same observation was suggested by Aklilu et al. [45].

Lambs are susceptible to different rotavirus serotypes during their first week of life [43]. Rotavirus-associated enteritis in lambs has been

the subject of numerous studies; the frequency of infection ranges from 2.1 % in Spain [23] and 5.3 % in Turkey [44] to 25 % in India [52]. In our study, the prevalence encountered (12.88 %) falls between these prevalences.

In the present study, rotavirus was more observed in lambs less than 7 days old (35.48 %). The quantitative and qualitative insufficiency of colostrum can be incriminated, especially since double births are frequent in ewes [53].

Several studies have shown high morbidity (75–100 %) in outbreaks of neonatal diarrhea in lambs [54]. In the present study, rotavirus was more prevalent in diarrhoea than non-diarrhoeal lambs, which is consistent with Kaminjolo et al. [55].

Rotavirus was isolated from 30 % of the farms in our study, which is lower compared to 46 % found in Scotland [52].

Coronavirus has been reported to play a role in the etiology of diarrhoea in association with rotavirus [56,57]. In the present study, coronavirus was detected in 3.57 % of lambs, which was lower than reported by other authors [44,58].

In addition, a survey of small ruminants in Spain failed to detect coronaviruses in diarrhoeal neonates [23].

Many enteric pathogens can exist simultaneously with cryptosporidium infection in the same animal [23]. Although infections with *C. parvum* alone were recorded, mixed infections with the other enteric pathogens tested were more frequent. As demonstrated also, single infections with *E. coli* K99, rotavirus and coronavirus may be associated with diarrhoea [59]. For mixed infections, *C. parvum* has been variably detected with *E. coli* K99, rotavirus and coronavirus. These mixed infections are common and are generally thought to increase the severity of the diarrhea when they occur [59].

In our study, mixed infections were observed between *C. parvum* and *E. coli* K99, rotavirus or coronavirus and no other form of association was found. This suggests that *C. parvum* is a primary agent in neonatal diarrhoea in lambs and that other agent is synergistic. The same observation has been reported by other authors [60,61].

5. Conclusion

The present study has documented *C. parvum*, as well as *E. coli* K99, rotavirus, and coronavirus in neonatal lambs from Algeria. These enteropathogens were observed in lambs with or without diarrhea. *C. parvum* was the most common, especially in lambs with diarrhea. For better prevention of neonatal diarrhea in lambs, special attention should be given to the first colostrum feeding, hygiene of the farm, prevention and control measures. To better understand the epidemiology of

neonatal diarrhoea in lambs in Algeria, more extensive and nationwide studies will be necessary.

Declaration of Competing Interest

The authors report no declarations of interest.

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