Enteropathogens associated with neonatal calves diarrhea in Tiaret area (Western Algeria)

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Abstract

The aims of this study were to assess the prevalence of rotavirus, coronavirus, Cryptosporidium spp. and E. coli strains associated with neonatal diarrhea in one-month-old calves. Fifty fecal samples collected from diarrheic calves were examined bacteriologically for E. coli, and by Sandwich ELISA screening for rotavirus, coronavirus, Cryptosporidium spp. and E. coli F5 strain. Cryptosporidium spp. was the dominant enteropathogen followed by E. coli. The E. coli fimbrial adhesin (F5, F17, F41) and the afimbrial adhesin CS31A were determined by slide agglutination using specific antiserum, and the standardized disc diffusion assay was used to estimate the antimicrobial sensitivity. Bacterial culture identified E. coli in 62% of the samples. The fimbriae F17 were detected in 29% followed by fimbriae F5 (26%), and afimbrial adhesin CS31A (9.68%) in E. coli isolates. 68.75% of E. coli. was shown to be multidrug resistant. The present study demonstrates a high multidrug-resistant strains of E. coli isolated from the neonatal calves’ diarrhea, and the clearly higher frequency of Cryptosporidium spp. in comparison with the other enteropathogens.

Keywords: Neonatal calves diarrhea, E. coli, fimbriae, afimbrial adhesion, Cryptosporidium spp.

Introduction

Neonatal calf diarrhea (NCD) is an important disease of young calves and has a complex etiopathogenesis (Courea et al., 2015). Calves are at greatest risk of developing diarrhea within the first month of life, and the incidence of diarrhea decreases with age (Gebregiorgis and Tessema, 2016). In the dairy industry, approximately 50 % of deaths in one-month-old calves have been attributed to diarrhea (Mohamed et al., 2017).

Enterotoxigenic Escherichia coli (ETEC), rotavirus, coronavirus and Cryptosporidium are the four major pathogens associated with NCD (Shams et al., 2010). Among E. coli associated with NCD, the major virulence factor is the F5 (de Verdier et al., 2012). Strain with this fimbrial adhesin is usually isolated form calf with diarrhea between one and five days of age (Umpiérrez et al., 2016). Nevertheless, strains with F17, F41 fimbrial adhesin and afimbrial adhesin CS31A of E. coli have also been isolated (Umpiérrez et al., 2016 and Nguyen et al., 2011).

The complex etiology, the absence of pathognomonic signs and lesions, the presence of asymptomatic infections, the interaction between intrinsic and extrinsic factors that predispose the host to infection by neonatal diarrhea lead to difficulty in clinical diagnosis of this syndrome (Athanasios et al., 1994). For this reason, laboratory diagnosis is necessary. Many diagnostic techniques that may be used to detect the enteropathogens in fecal samples include isolation and direct electron microscopy, immuno-electron microscopy, as well as assays to detect antigens (latex agglutination and ELISA, polyacrylamide gel electrophoresis), and nucleic acid (reverse transcription polymerase chain reaction) (Mayameei et al., 2010). Commercial Ag-ELISA kits for detecting bovine rotavirus type A, bovine coronavirus, E. coli K99, and/or Cryptosporidium parvum in faecal samples are available. Ag-ELISAs are well known for rapid turnaround, high-throughput testing, plug-in-and-play capability, and portability (Cho et al., 2014).

Diarrhea is one of the most important causes of morbidity and mortality, leading to economic losses due to the cost of treatment, prophylaxis, increased susceptibility to other infections, reduced growth rates, and death of calves (Rocha et al., 2017). However, antimicrobial therapy is the primary control approach for decreasing morbidity.
and mortality in animals infected with diarrhoeagenic bacteria (Pourtaghi et al., 2016). In addition, antimicrobial agents are used in chemoprophylaxis, chemotherapy and as growth promoters in the livestock industry (Roopnarine et al., 2009). However, the broad use of antimicrobials in animal production may lead to the emergence and dissemination of antimicrobial-resistant bacterial pathogens, which without doubt pose a potential threat to human public health (Du et al., 2005). The problem of drug resistance is not restricted to pathogenic bacteria; it also involves the commensal bacterial flora, which may become a major reservoir of resistant strains (Erb et al., 2007). Thus, the purpose of this study conducted in Western Algeria (Tiaret) was the estimation of the prevalence of rotavirus group A, bovine coronavirus, Cryptosporidium spp., and E. coli associated with NCD.

Material and Methods

Fecal samples
Fifty fecal samples were obtained from both sexes of calves with diarrhea, aged less than 30 days, who had not received prior treatment with antibiotics during calving period. The collected samples were sent out to the laboratory in ice-cooled containers and stored at 4 °C until bacteriological examination and at −20 °C until analyzing by ELISA test.

Bacteriological examination
Immediately after receipt at the microbiology laboratory, the samples were inoculated with a swab on Mac Conkey agar (Biochem, Quebec) and incubated for 18-24 hours at 37 °C. A lactose-positive colony from the fecal culture from each calf was selected and confirmed biochemically by API 20E (BioMérieux, French).

All E. coli strains previously identified by API 20E were inoculated onto the surface of Minca IsoVitalex media (BioVac laboratory, French), and then slide agglutination was used with the specific antisera (BioVac laboratory, French) to detect E. coli fimbrial adhesion F5, F17, F41 and afimbrial adhesin CS31A.

ELISA test
Fecal samples (n=50) were tested for the presence of rotavirus, coronavirus, Cryptosporidium spp, and E. coli F5 by an antigen ELISA (BIO K 348 - Multiscreen AgELISA Calf digestive / sandwich, Bio-X Diagnostics Sprl, Belgium) according to the manufacturer’s instructions.

These samples are stratified into four age-groups. Briefly, 10 of the calves included in this study were 1-7 d old, and 15, 18 and 7 calves were 8-14, 15-21 and 22-30 d old, respectively.

Antibiotic susceptibility test
Susceptibility of E. coli isolates to a panel of antimicrobial agents was determined by the standardized disc diffusion assay on Mueller-Hinton agar (BioMérieux, French) using commercial antimicrobial susceptibility discs according to the recommendations of the Standardization of antimicrobial susceptibility testing in the veterinary medicine (MoARD, 2008 and 2011). The tested antibiotics and their corresponding disc concentrations were as follows: amoxicillin+ acid clavulanic (AMC: 20/10 µg), ampicillin (AM: 10 µg), gentamicin (GN: 10 µg), tetracycline (TE: 10 µg), colistin (CT: 10 µg), trimethoprim/sulfamethoxazole (STX: 1.25/23.75 µg), ofloxacin (OFX: 5 µg) and cefotaxime (CTX: 30 µg).

Statistical Analysis
The chi-square test was used at 95 % significance to estimate the differences among the proportion of fecal samples that tested positive or negative for the enteropathogens studied and the age group of animals with diarrhea.

Results

Typing of isolated E. coli
In this study, E. coli was the most isolated bacterium (62%). Among these isolates, 64.52% belonged to strains possessing fimbriae F5, F17 and afimbrial adhesin CS31A (Figure 1).

Elisa test
The most frequently detected enteropathogens were Cryptosporidium spp. (40%) followed by E. coli F5 (16%), coronavirus (10%) and rotavirus (8%) (Table 1). The proportions of positive samples for the four enteropathogens during the four weeks of the first month of their lives were summarized in Table 1. All the four investigated age groups were positive to the enteropathogens. Nevertheless, the 8-14 d old calves showed higher frequency (80%) to enteropathogen infection compared to the other three age groups, but no significant difference was found by statistical analysis.

Antimicrobial resistance
High antimicrobial resistance was observed from tested isolates and E. coli against amoxicillin + clavulanic acid, ampicillin, tetracycline and trimethoprim/sulfamethoxazole. On the other hand, there was a high sensitivity of these strains to cefotaxime, colistin, gentamicin and ofloxacin (Fig. 2). Twenty-two isolates (68.75%) of E. coli were resistant to three or more class of antimicrobials.
Enteropathogens associated with neonatal calves diarrhea in Tiaret area (Western Algeria)

Figure 1: Typing of isolated E. coli

Table 1. Detection of four tested enteropathogens in the different age groups of diarrheic calves

<table>
<thead>
<tr>
<th>Enteropathogen(s) detected</th>
<th>Age of diarrheic calves (days)</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-7d (n=10)</td>
<td>8-14d (n=15)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>None</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Cryptosporidium only</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>E. coli F5 only</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Coronavirus only</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Rotavirus only</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cryptosporidium + E. coli F5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cryptosporidium + Coronavirus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cryptosporidium + Rotavirus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. coli F5 + Rotavirus</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure 2: Antimicrobial resistance of strains of E. coli

R: Resistant, I: Intermediate, S: Sensitive

Discussion and conclusions

Diarrhea is a complex syndrome, resulting from the interaction of several infectious agents (alone or in combination), host factors and non-infectious factors (environment, management practices and nutrition). Several infectious agents are implicated in its etiology. However, *Salmonella* spp., pathogenic *Escherichia coli*, coronavirus and rotavirus are the most frequent pathogens associated with neonatal calves diarrhea (COURA et al., 2015).

Out of 50 fecal samples collected from diarrheic calves, 31 (62%) samples were found positive for *E. coli* after biochemical characterization. Compared with present study, higher frequency in *E. coli* was observed by Shahrani et al. (2014) in Iran 76.45% and Younis et al. (2009) (87.72%) in Egypt. However Picco et al. (2015) and Herrera-Luna et al. (2009) mentioned lower rates with respective frequency of 30.1% and 18.9%.

ETEC infection is the most common type of colibacillosis in young animals such as calves (SHAMS et al., 2012). This result is in agreement with our study (64.52%) and it was higher than those cited by Pourtaghi et al. (2016), Shahrani et al. (2014) and Younis et al. (2009) (11.3%, 28.4%, 10.36%, respectively).

F17 fimbriae, known as FY or Att25, are prevalent in isolated *E. coli* strains in calves with diarrhea or septicemia (Nguyen et al., 2011). In this study, 29% of isolated *E. coli* strains carried F17 fimbriae. Similar result (28.7%) was reported by Umphierrez et al. (2016).

The prevalence of K99 fimbriae (F5) was 26%. This result is similar to that reported by İçen et al. (2013). However, many previous studies mentioned a lower prevalence ranging from 5.3% to 17.4% (Lacrécourt et al., 2016; Shems et al., 2012; Akam et al., 2011 and Izzo et al., 2011).

CS31A is defined as an afimbrial adhesin of *E. coli*, immunologically related to the F4 fimbria (formerly K88), and also plasmid encoded. The CS31A antigen was first recognized as a capsule-like surface protein around the bacteria, and it has been extensively studied in septicemia and enterotoxigenic *E. coli* strains. CS31A is usually expressed with other fimbria antigens. Particularly, it has been reported that F17 fimbria and CS31A are highly associated (Umphierrez et al., 2016). In our study, the strains carrying this antigen were detected with a prevalence of 9.68%. These prevalence rates were lower than those cited by Umphierrez et al. (2016) and Lacrécourt et al. (2016) (17.3% and 77.3%, respectively).

In the present study, no detection of fimbriae F41 was observed. A similar finding was reported by Lacrécourt et al. (2016). Whereas Nguyen et al. (2011) and Ok et al. (2009) showed the prevalence rates of *E. coli* F41 from 4.93% and 18.9% respectively.

The differences in prevalence and frequency rates among the studies may be attributed to regional variations, management and hygienic conditions, the age of the animals, vaccination of dam, colostrum intake, or to the fact that the analysis of fimbriae in the previous reports was determined by different diagnostic methods (Picco et al., 2015 and İçen et al., 2013).

*Cryptosporidium* spp. is one of the most important causative agents of acute diarrhea, especially in young calves (Gillhuber et al., 2014). In our study, *Cryptosporidium* spp. was the predominant enteropathogen associated with calves’ diarrhea. The incidence of this agent is 40% either alone or in combination with other causative agent. Our result is in agreement with the results reported by İçen et al. (2013) (47.8%) and Langoni et al. (2004) (38.9%), but lower to those cited by Izzo et al. (2011) (58.5%).

In our study, *E. coli* F5 was the second predominant enteropathogen associated with calves’ diarrhea with the prevalence rate of 16% (detected by ELISA). Similarly, Akam et al. (2011) and Izzo et al. (2011) have cited the prevalence rates of 15.12% and 17.4%, respectively. Picco et al. (2015) in Argentina and Younis et al. (2009) in Egypt observed the prevalence of 12.9% and 10.36%, respectively.

Rotavirus and coronavirus are the most common viral causes of NCD (Mayameei et al., 2010). In this study, coronavirus was detected in 10% of NCD. This result is similar to that reported by Perez et al. (1998) in Costa Rica (9%), but lower than those recorded by Selles et al. (2014) in Algeria (20.73%) and Izzo et al. (2011) in Australia (21.6%).

The rotavirus infections were detected at less prevalence rates (8%). This finding corroborates with the result noticed by Perez et al. (1998) in Costa Rica (7%). Nonetheless, higher prevalence have been reported in Egypt by Mohamed et al. (2017) (46%), Selles et al. (2014) in Algeria (14.63%), Izzo et al. (2011) in Australia (79.9%) and Ok et al. (2009) in Turkey (18.2%).

The differences in detection of enteropathogens may be attributed to the different diagnostic methods used (Ok et al., 2009), farm management practices, hygienic status (Mayameei et al., 2010; Ok et al., 2009), environmental condition, and geographical locations (climate condition) (Selles et al., 2014). Moreover, the increasingly frequent use of vaccines against rotavirus, coronavirus and *E. coli* F5 can explain the reduced rates of virus prevalence compared to the previous study conducted in the same area.

No enteropathogens were detected in 17 calves (34%). This rate was higher than that observed by Ok et al. (2009), Izzo et al. (2011) and İçen et al. (2013). Probable explanations for this lack of detection may be related to the non-infectious origin of diarrhea (nutritional diarrhea) or infectious diarrhea whose etiological agents were not targeted by this study.

This study shows that the frequency of infection is higher in age group 8-14 d old (80%). This age bracket is considered as the most susceptible to infection by *Cryptosporidium* spp. (60%). Similar results were archived by Gillhuber et al. (2014). The probable explanation for this finding may be related to either a poor and/or no absorption of colostrum and weakening of passive immunity. While the first three age groups were characterized by the highest fre-
quency of diarrhea, later on, the cases of diarrhea progressively decreased. The same findings have been reported in previous studies (Coura et al., 2015; Gebregiorgis and Tessema, 2016; Mayameei et al., 2010). This can be explained by increase in natural resistance against infection.

Several previous studies have reported the antimicrobial resistance of *E. coli* strains isolated from NCD. Higher antimicrobial resistance towards the several antibiotics were archived by Lacroute et al. (2016) and Shahrani et al. (2014). However, Rigobelo et al. (2006) cited low antimicrobial resistance. In Algeria, Akam et al. (2007) reported antimicrobial resistance of *E. coli* F5 strains to ampicillin, tetracycline and chloramphenicol.

According to literal terms, multidrug-resistant (MDR) means “resistant to more than one antimicrobial agent”, but the medical community has agreed upon no standardized definitions for MDR yet. Many definitions are being used in order to characterize patterns of MDR in Gram-positive and Gram-negative organisms. One of the methods used by various authors and authorities to characterize organisms as MDR is based on in vitro antimicrobial susceptibility test results, when they test “resistance to multiple antimicrobial agents, classes or subclasses of antimicrobial agents”. The definition most frequently used for Gram-positive and Gram-negative bacteria is “resistant to three or more antimicrobial classes” (Magiorakos et al., 2012).

Based on this definition, the MDR of *E. coli* strains studied was 68.75%. This finding corroborates with the result noticed by Souto et al. (2016) (78.68%). Additionally, these results are in agreement with those observed by Rigobelo et al. (2006) in Brazil who reported 80% multi-resistance for *E. coli* isolates.

This higher rate of antimicrobially resistant strains isolated from NCD could be explained by increase in drug usage (Roopnarine et al., 2009). De Verdier et al. (2012) incriminated the calf feed of milk or colostrum from cows treated with antibiotics as a possible source of antibiotic resistance.

The results of this survey highlight a number of important facts in regard to enteropathogens associated with NDC. They provide valuable insight into the pathogen associated with outbreaks of diarrhea in dairy calves across Tiaret area. *Cryptosporidium* spp. was the most prevalent enteropathogen identified in calves aged less than 30 days. This is the first report on the presence of fimbrial adhesin F5, F17 and afimbrial adhesin CS31A in *E. coli* strains isolated from NCD cases in Tiaret area (Algeria). This study indicates a high frequency of antimicrobial resistance among *E. coli* strains isolated from NCD against antibiotics frequently used in Algerian veterinary medicine.

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**Conflict of interest statement**

We declare that we have no conflict of interest.

**References**


Enteropathogens associated with neonatal calves diarrhea in Tiaret area (Western Algeria) —102/103


**Enteropatogeni uzročnici dijareje kod neonatalne teladi u području Tiareta (zapadni Alžir)**

**Apstrakt**

Cilj istraživanja jeste određivanje prevalencije rotavirusa, koronavirusa, *Cryptosporidium* spp. i sojeva *E. coli* povezanih sa neonatalnom dijarejom kod jednomjesečne teladi. Pedeset uzoraka fecesa prikupljenih od teladi sa dijarejom je bakteriološki ispitano na *E. coli*, i obavljen je skrining Sandwich ELISA metodom na rotavirus, koronavirus, *Cryptosporidium* spp. i *E. coli*, soj F5. Dominantan enteropatogen je *Cryptosporidium* spp., slijedi *E. coli* F5. Korištenjem specifičnog antiseruma metodom aglutinacije su određeni fimbrijalni adhezin *E. coli* (F5, F17, F41) i afimbrijalni adhezin CS31A. Da bi se procijenila antimikrobna senzitivnost, urađena je i standardna disk-difuziona metoda antibiograma. Bakterijske kulture su u 62% uzoraka dokazale prisustvo *E. coli*. U izolatima *E. coli* su naviše dokazani sojevi F17 u 29% slučajeva, zatim F5 (26%) i CS31A (9.68%). Ukupno 68.75% izolata *E. coli* se pokazalo multirezistentnim na testirane antibiotike. Naše istraživanje pokazuje da su iz fecesa neonatalne teladi sa dijarejom izolirani izrazito rezistentni sojevi *E. coli*, i da je učestalost *Cryptosporidium* spp. u odnosu na druge enteropatogene znatno veća.

**Ključne riječi:** dijareja neonatalne teladi, *E. coli*, fimbrie, afimbrijalna adhezija, *Cryptosporidium* spp.