

## Role of enteric pathogens in the aetiology of neonatal diarrhoea in lambs and goat kids in Spain

M. MUÑOZ, M. ÁLVAREZ, I. LANZA AND P. CÁRMENES

Departamento de Sanidad Animal (Enfermedades Infecciosas y Epidemiología), Facultad de Veterinaria, Universidad de León, Campus de Vegazana, E-24071 León, Spain

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### SUMMARY

Faeces samples from diarrhoeic and non-diarrhoeic lambs and goat kids aged 1–45 days were examined for enteric pathogens. *Cryptosporidium parvum* was detected in both diarrhoeic lambs (45%) and goat kids (42%) but not in non-diarrhoeic animals. F5<sup>+</sup> (K99<sup>+</sup>) and/or F41<sup>+</sup> *Escherichia coli* strains were isolated from 26% and 22% of the diarrhoeic lambs and goat kids, respectively, although these strains, which did not produce enterotoxins ST I or LT I, were found with similar frequencies in non-diarrhoeic animals. A F5<sup>-</sup>F41<sup>-</sup>ST I<sup>+</sup> *E. coli* strain was isolated from a diarrhoeic lamb (0.6%). Verotoxigenic *E. coli* was isolated from both diarrhoeic and non-diarrhoeic lambs (4.1% and 8.2%, respectively) and there was no association between infection and diarrhoea. The prevalence of group A rotavirus infection in diarrhoeic lambs was very low (2.1%). Groups A and B rotaviruses were detected in three (8.1%) and five (13.5%) diarrhoeic goat kids from two single outbreaks. Group C rotaviruses were detected in four non-diarrhoeic goat kids. An association of diarrhoea and infection was demonstrated only for group B rotavirus. *Clostridium perfringens* was isolated from 10.8% of the diarrhoeic goat kids but not from non-diarrhoeic goat kids or lambs. *Salmonella arizonae* was isolated from a diarrhoeic goat kid (2.7%) and the clinical characteristics of the outbreaks where these two latter enteropathogens were found different from the rest. Picobirnaviruses were detected in a diarrhoeic lamb. No coronaviruses were detected using a bovine coronavirus ELISA. No evidence was found of synergistic effect between the agents studied. Enteric pathogens were not found in four (8.7%) and three (20%) outbreaks of diarrhoea in lambs and goat kids, respectively.

### INTRODUCTION

Diarrhoea is an important problem in young domestic animals although its aetiology is not well understood since several agents may be involved concurrently. Moreover, many of these agents are capable of infecting the host without inducing the clinical illness. The aetiology and epidemiology of the syndrome have been extensively studied in cattle and pigs, however, very few studies have been performed to investigate the enteric pathogens that cause diarrhoea in newborn

lambs and goat kids. [1] In lambs and goat kids, rotavirus, enterotoxigenic *Escherichia coli* (ETEC) and *Cryptosporidium parvum* are considered among the most prevalent organisms associated with diarrhoea [2–5]. *Clostridium perfringens* and *Salmonella* species are also thought to play an important role [6], but few cases in which any of these pathogens were involved have been reported. Other agents, such as enteroviruses, astroviruses, coronaviruses or *E. coli* bearing virulence attributes different from those typical of ETEC (verotoxigenic, F17<sup>+</sup> or attaching-

effacing *E. coli*), have been found in diarrhoeic and healthy lambs and goat kids but the pathogenic significance for these species is unknown [3, 7–11].

In Spain, the 'neonatal diarrhoeic syndrome' is one of the major health problems affecting sheep and goats. Because in most farms parturition takes place indoors, hypothermia and predation are less frequent while infectious diseases are the main cause of newborn losses, gastrointestinal infections being one of the most common. In a previous study carried out in a small area of Northern Spain, neonatal diarrhoea was the most frequent health problem about which sheep farmers consulted veterinarians, representing 23% of the consultations during 1993 [12]. Thus, the aim of this study was to estimate the prevalence of different enteropathogens in young lambs and goat kids in North West Spain, and to assess their potential role in the aetiology of the 'neonatal diarrhoeic syndrome' of both species.

## MATERIALS AND METHODS

### Farms

Faeces samples were collected between 1988 and 1992 from 50 sheep and 16 goat farms located in Castilla-León (North West Spain), a region with a sheep and goat population of about 5 million and 300 000 animals, on 25 000 and 8000 farms, respectively. The mean herd size was 180 animals for sheep and 40 for goats. Both species are mainly reared under semi-extensive husbandry but while sheep farms produce milk, meat or both, most goat farms produce only meat. There may be one or two breeding seasons a year. Most animals are born indoors and weaned when aged 1–3 months. Newborn animals are generally kept indoors until weaning while their mothers go out to graze during the day.

### Faecal samples

Faecal samples were collected from 190 diarrhoeic lambs in 46 outbreaks of neonatal diarrhoea (mean 4.1 per outbreak, range 1–24). Forty additional samples from non-diarrhoeic animals were collected from six farms, four of which had no neonatal diarrhoea problem (mean 6.6, range 1–16). Faecal samples were collected from 36 diarrhoeic goat kids from 15 outbreaks of neonatal diarrhoea (mean 2.6, range 1–12). Ninety-nine additional samples were collected from non-diarrhoeic goat kids on three

farms, one of which had not experienced a diarrhoea outbreak (mean 33, range 17–51).

Samples were either sent to our laboratory by the practitioner or collected by us from the farm. The clinical characteristics such as duration of diarrhoea, faecal consistency and colour, presence of other signs and percentage of affected (morbidity) and dead (mortality) animals were recorded for each outbreak.

Swabs of rectal faeces were taken from live animals and placed immediately in 1 ml of 0.1 M sterile phosphate-buffered saline (PBS). Faeces or intestinal contents were obtained from the intestinal tract of dead animals and diluted 1:10 in PBS. Samples were refrigerated at their arrival to the laboratory, a maximum of 6 h after their collection.

### Detection of enteric viruses

Faecal samples were examined for the presence of rotavirus using four methods: a double antibody sandwich ELISA, two commercial latex agglutination tests (Slidex Rotakit II, Biomérieux, France; Rotalex, Orion Diagnostica, Finland), and by polyacrylamide gel electrophoresis (PAGE) [13]. Only 55 ovine and 63 caprine samples were assayed using the latex agglutination tests. Any sample scored as positive in any of the four tests was considered as containing rotavirus. The first three tests are specific of group A rotaviruses while PAGE is capable of detecting non-group A rotaviruses and other dsRNA viruses such as picobirnaviruses.

Faeces were examined for coronaviruses using a blocking ELISA developed for detecting bovine coronaviruses. The procedure described by De Leeuw and colleagues [14] was followed with some modifications. Briefly, polystyrene microtiter plates were coated with calf anti-bovine coronavirus IgG (provided by Dr DeLeeuw) as capturing antibody, and faeces samples, diluted in PBS 0.05% Tween-80, were added in duplicate. One of the wells was blocked using a calf anti-bovine coronavirus serum. After incubation and washing of the plates with PBS Tween-80, non-blocked coronavirus antigen was detected with peroxidase-labelled bovine anticoronavirus IgGs. The substrate chromogen mixture was 0.005% H<sub>2</sub>O<sub>2</sub> and 1 mg/ml 5-aminosalicylic acid diluted in phosphate-EDTA buffer.

### Detection of enteric bacteria

Faeces were inoculated onto McConkey agar (Difco, USA) and both lactose-fermenting and non-

fermenting colonies, with different morphology were subcultured onto the same agar. They were speciated using the API 20E system (Biomérieux, France) and stored at  $-80^{\circ}\text{C}$  in nutrient broth with 30% glycerol. To detect the presence of virulence factors in *E. coli*, strains were thawed and cultured on Minca agar [15] in order to encourage the expression of F5 and F41 fimbriae, which were detected using a monoclonal antibody based latex agglutination test (Fimbrex, CVL, UK). Strains that agglutinated the test and the control reagents were subcultured in tryptone soya broth (TSB) (Oxoid, USA), incubated in an orbital shaker (200 rpm) at  $37^{\circ}\text{C}$  for 18–24 h, and the agglutination test repeated. Strains that agglutinated only the positive reagent were considered positive. To investigate the production of thermostable enterotoxin I (ST I) and thermolabile enterotoxin I (LT I) two commercial kits were used. *E. coli* ST EIA (Oxoid, UK) is a competition ELISA that specifically detects ST I, and VET-RPLA (Oxoid, UK) is a passive latex agglutination test that detects the enterotoxin of *Vibrio cholerae*, which is antigenically very similar to LT I. Strains were cultured in 4 ml of CAYE broth [16] and incubated in an orbital shaker (200 rpm) at  $37^{\circ}\text{C}$  for 18–24 h. One ml of broth was centrifuged at 900 g for 20 min and the supernatant was used as the test sample for the ST EIA kit. The remaining 3 ml were treated with 10000 IU/ml of polymyxin B for 4 h at  $37^{\circ}\text{C}$ , centrifuged at 900 g for 20 min and the supernatant used as the test sample for the VET-RPLA kit. The production of verotoxins (VT), cytotoxic necrotizing factors (CNF) and LT was investigated using the Vero cell assay. Four colonies were cultured in a 200 ml Erlenmeyer flask containing 20 ml of TSB. After 18–24 h of incubation at  $37^{\circ}\text{C}$  in an orbital shaker, the culture was centrifuged 10 min at 2300 g and supernatant filtered through  $0.45\ \mu\text{m}$  pore size membrane (Millipore Corp., USA). Vero cell assays were performed using monolayers grown in Minimal Essential Medium plus 10% foetal calf serum in 24 well plates. Growth medium was discarded and fresh medium plus  $50\ \mu\text{l}$  of the bacterial filtrate were added to each well. Cells were incubated at  $37^{\circ}\text{C}$  in 5%  $\text{CO}_2$  and observed in a phase contrast inverted microscope for the appearance of a cytotoxic effect every 24 h for 3 days. Strains P3 (VT<sup>+</sup>), S5 (CNF 2<sup>+</sup>) and H296 (LT I<sup>+</sup>), provided by Dr Wray (CVL, Weybridge, UK), were used as positive controls. All *E. coli* strains bearing at least one virulence factor were considered 'potentially pathogenic'.

The presence of *Cl. perfringens* was determined by

microscopic examination of Gram-stained faecal smears. Faecal samples in which Gram positive bacilli were observed and cases in which *Cl. perfringens* was suspected clinically, were cultured in cooked meat broth (Difco, USA) as an enrichment medium and subcultured onto trypticase sulfite neomycin agar (Biomérieux, France), as a differential medium. Plates were incubated anaerobically for 24 h and black colonies were considered suspicious of *Cl. perfringens* and inoculated onto API 20A galleries (Biomérieux, France) for confirmation.

#### Detection of *Cryptosporidium parvum*

Faecal smears were stained by the Ziehl-Neelsen method modified by Henriksen and Polenz [17] and examined microscopically for *C. parvum* oocysts.

Due to insufficient amount of faecal material some of the samples could not be assayed for the detection of all enteropathogens.

#### Statistical analysis

The prevalence of the different enteropathogens in each animal species between diarrhoeic and non-diarrhoeic animals and the detection rates of different mixed infections in both groups of animals were compared using the  $\chi^2$  or the Fisher's exact test (two-tailed) at  $\alpha = 0.05$ .

In order to assist the assessment of the pathogenic role of the agents, the within-flock prevalence of infection was calculated for each enteropathogen as the number of positive diarrhoeic animals/number of diarrhoeic animals tested per outbreak. Only those outbreaks where four or more diarrhoeic samples had been analysed were included.

## RESULTS

The principal results are summarized in Table 1. *C. parvum* and potentially pathogenic *E. coli* were the most frequently detected agents in both species. *C. parvum* was found in 45% of diarrhoeic lambs and 42% of diarrhoeic goat kids. No *C. parvum* oocysts were detected in non-diarrhoeic animals and a highly significant association was found between infection and diarrhoea in both lambs and goat kids ( $\chi^2 = 24.4$ ,  $P < 0.001$  and  $\chi^2 = 19.4$ ,  $P < 0.001$ , respectively).

*E. coli* bearing at least one virulence factor was isolated both from diarrhoeic lambs (30%) and goat kids (22%) with fimbriated *E. coli* being the most

Table 1. *Enteropathogens detected in diarrhoeic and non-diarrhoeic animals and in outbreaks of diarrhoea in lambs and goat kids*

|                                 | No. positives/no. tested (%) |        |                |        |           |        |            |        |                |        |           |        |
|---------------------------------|------------------------------|--------|----------------|--------|-----------|--------|------------|--------|----------------|--------|-----------|--------|
|                                 | Lambs                        |        |                |        |           |        | Goat kids  |        |                |        |           |        |
|                                 | Animals                      |        |                |        |           |        | Animals    |        |                |        |           |        |
|                                 | Diarrhoeic                   |        | Non-diarrhoeic |        | Outbreaks |        | Diarrhoeic |        | Non-diarrhoeic |        | Outbreaks |        |
| Group A rotavirus               | 4/190                        | (2.1)  | 1/40           | (2.5)  | 3/46      | (6.5)  | 3/37       | (8.1)  | 15/98          | (15.3) | 1/14      | (7.1)  |
| Group B rotavirus               | 0/190                        | (0)    | 0/40           | (0)    | 0/46      | (0)    | 5/37       | (13.5) | 1/98           | (1)    | 1/14      | (7.1)  |
| Group C rotavirus               | 0/190                        | (0)    | 0/40           | (0)    | 0/46      | (0)    | 0/37       | (0)    | 4/98           | (4)    | 0/14      | (0)    |
| Total rotavirus                 | 4/190                        | (2.1)  | 1/40           | (2.5)  | 3/46      | (6.5)  | 8/37       | (21.6) | 19/98          | (19.4) | 2/14      | (14.2) |
| Coronavirus                     | 0/80                         | (0)    | 0/30           | (0)    | 0/18      | (0)    | 0/8        | (0)    | 0/4            | (0)    | 0/5       | (0)    |
| Picobirnavirus                  | 1/190                        | (0.5)  | 0/36           | (0)    | 1/46      | (2.2)  | 0/37       | (0)    | 0/98           | (0)    | 0.14      | (0)    |
| Fimbriated <i>E. coli</i>       | 44/171                       | (25.7) | 16/36          | (44.4) | 25/46     | (54.3) | 8/37       | (21.6) | 1/6            | (16.7) | 5/14      | (35.7) |
| STI <sup>+</sup> <i>E. coli</i> | 1/171                        | (0.6)  | 0/36           | (0)    | 1/46      | (2.2)  | 0/37       | (0)    | (0/6)          | (0)    | 0/14      | (0)    |
| VT <sup>+</sup> <i>E. coli</i>  | 7/171                        | (4.1)  | 3/36           | (8.3)  | 6/46      | (13)   | 0/37       | (0)    | 0/6            | (0)    | 0/14      | (0)    |
| AT <sup>+</sup> <i>E. coli</i>  | 2/171                        | (1.2)  | 1/36           | (2.8)  | 3/46      | (6.5)  | 0/37       | (0)    | 0/6            | (0)    | 0/14      | (0)    |
| Total <i>E. coli</i>            | 52/171                       | (30.4) | 18/36          | (50)   | 28/46     | (60.8) | 8/37       | (21.6) | 1/6            | (16.7) | 5/14      | (35.7) |
| <i>Salmonella</i> spp           | 0/171                        | (0)    | 0/36           | (0)    | 0/46      | (0)    | 1/37       | (2.7)  | 0/6            | (0)    | 1/14      | (7.1)  |
| <i>Cl. perfringens</i>          | 0/185                        | (0)    | 0/33           | (0)    | 0/46      | (0)    | 4/37       | (10.8) | 0/6            | (0)    | 3/15      | (20)   |
| <i>C. parvum</i>                | 82/183                       | (44.8) | 0/34           | (0)    | 30/46     | (65.2) | 15/36      | (41.7) | 0/43           | (0)    | 6/15      | (40)   |

prevalent (Table 1). F41 was detected more frequently than F5 with 12.4% and 8.2% of the strains isolated, respectively, from lambs and goat kids expressing F41, 8.3% and 6.4% expressing F5 and F41, and 7% and 4.1% expressing F5. None of the fimbriated strains produced enterotoxin. Only one ovine strain, isolated from a diarrhoeic lamb, was ST I<sup>+</sup>. It did not express any other virulence factor. LT I was not detected with the latex agglutination test, but three of the ovine strains, isolated from three outbreaks, produced a toxin that induced a cytopathic effect in Vero cells similar to that of strain H296 (LT I<sup>+</sup>). This atypical toxin (AT) was the only virulence factor detected in these three strains. Eleven ovine strains, isolated from 10 samples in 6 outbreaks, induced a cytotoxic effect similar to that of P3 strain (VT<sup>+</sup>) and were considered verotoxigenic, 7 were also fimbriated. None of the strains induced a cytotoxic effect on Vero cells similar to that of S5, a CNF 2<sup>+</sup> strain. Potentially pathogenic *E. coli* was detected in 30% of diarrhoeic and 50% of non-diarrhoeic lambs and in 22% of diarrhoeic and 17% of non-diarrhoeic kids (Table 1). No significant differences were found in the prevalence of fimbriated, ST I<sup>+</sup>, AT<sup>+</sup>, VT<sup>+</sup> strains between diarrhoeic and non-diarrhoeic animals, except that the prevalence of fimbriated *E. coli* in lambs was significantly higher ( $P < 0.05$ ) in non-diarrhoeic than in diarrhoeic lambs.

Only 55 ovine and 63 caprine samples were assayed by latex agglutination to detect rotavirus antigen. Fifty-four percent of the lamb and 57% of the goat kid samples reacted non-specifically in the Rotalex kit with both the test latex and the control latex reagents giving inconclusive results. Non-specific reactions were observed with the Slidex-Rotakit II in 39% of the lamb and 1.4% of the goat kid samples. Rotavirus infection was detected in five lambs from three outbreaks (Table 1) and there was no statistical association between infection and diarrhoea. All five samples reacted in the ELISA specific for group A rotaviruses and had the characteristic electropherotype of this group. Rotavirus were detected more frequently in goat kids. Both group A and B rotaviruses were detected in diarrhoeic and non-diarrhoeic animals in two different diarrhoea outbreaks (Table 1). No association was found between infection and diarrhoea for group A rotavirus but a very significant association (Fisher's exact test  $P < 0.01$ ) was found for group B rotavirus. Both outbreaks have been described elsewhere [13, 18]. Group C rotaviruses were detected in four goat kids from a herd with no problem of diarrhoea.

*Cl. perfringens* was not detected in lamb faeces but it was isolated from diarrhoeic goat kids in three outbreaks (20%). Other enteropathogens were detected at a much lower prevalence. Two bands cor-



Table 2. Within-flock prevalence of enteropathogens in sheep and goat flocks where four or more samples from animals with diarrhoea were examined

|                                | Number of flocks | Mean within-flock prevalence (%) | Range (%) |
|--------------------------------|------------------|----------------------------------|-----------|
| Group A rotavirus              | 4                | 15.1                             | 5–26.9    |
| Group B rotavirus              | 1                | 19.3                             | —         |
| Group C rotavirus              | 1                | 23.5                             | —         |
| Fimbriated <i>E. coli</i>      | 15               | 28.1                             | 4.7–62.5  |
| VT <sup>+</sup> <i>E. coli</i> | 4                | 15.5                             | 6.1–42.8  |
| AT <sup>+</sup> <i>E. coli</i> | 2                | 4.5                              | 4.1–5     |
| <i>C. parvum</i>               | 12               | 52.6                             | 13.3–100  |

responding to the characteristic electropherotype of picobirnaviruses were detected by PAGE in the faeces of a diarrhoeic lamb. *Salmonella arizonae* was isolated from a goat kid with diarrhoea in an outbreak from which two more samples had been cultured. None of the lamb or goat kid samples reacted positively in the bovine coronavirus ELISA.

Mixed infections were detected in 15% of the lambs and 39% of the sheep flocks and in 14% of the goat kids and 33% of the goat herds, with *C. parvum* + *E. coli* being the most common combination. Group A rotaviruses + *C. parvum*, group A rotaviruses + *E. coli*, *E. coli* + *C. parvum* and *C. parvum* + *S. arizonae* were also detected. Three enteropathogens, *C. parvum*, *E. coli* and picobirnaviruses were detected in a diarrhoeic lamb. No statistically significant association was found between the presence of any mixed infection and diarrhoea in lambs and goat kids. No enteropathogens were detected in four (8.7%) and three (20%) outbreaks of diarrhoea in lambs and goat kids, respectively.

#### Prevalence of infection in the flock

The mean within-flock prevalences of infection were calculated for rotaviruses, fimbriated *E. coli*, AT<sup>+</sup> *E. coli*, verotoxigenic *E. coli* (VTEC) and *C. parvum* for those flocks in which at least four samples had been examined (Table 2). *C. parvum* was the enteropathogen with the higher mean within-flock prevalence of infection (53%) in the range 13–100%. The mean prevalence for the other enteropathogens was, in all cases, lower than 30% although fimbriated *E. coli* were detected in more than 40% of the samples in four outbreaks and VTEC in 57% of the samples in one outbreak.

#### Occurrence of enteropathogens in animals of different ages

Animals were grouped according to their age, and the prevalence of infection in each group calculated (Table 3). Similar prevalences were found for group A rotaviruses among all age groups of lambs, while in goats the excretion of group A rotavirus were most prevalent in the 6 to 10-day-old animals ( $\chi^2 = 10.99$ ;  $P < 0.001$ ). Group B and C rotaviruses were detected only in animals younger than 5 days, though the difference was significant only for group B rotaviruses (Fisher's exact test,  $P < 0.01$ ). *C. parvum* infection was more frequent in 6 to 10-day-old lambs ( $\chi^2 = 33.51$ ;  $P < 0.001$ ) while the highest prevalence was found in 10 to 15-day-old group for goat kids (Fisher's exact test,  $P < 0.05$ ). No significant differences were found in the age distribution for the other enteropathogens. Picobirnaviruses were detected in a 5-day-old lamb. ST I<sup>+</sup> *E. coli* was isolated from a 3-day-old lamb and AT<sup>+</sup> *E. coli* strains from lambs aged 7–15 days. *S. arizonae* was isolated from a 15 to 20-day-old goat kid and *Cl. perfringens* was isolated from three 5-day-old and one 10-day-old goat kid.

#### Clinical characteristics of the outbreaks

Most of the outbreaks had similar characteristics, with anorexia, prostration, absence of fever and diarrhoea as the main signs. However, when *C. parvum* was the only agent detected ( $n = 14$ ), morbidity was in the range 40–100% and more frequently 90–95%, especially at the end of the parturition season. Mortality was 0–65% and in most cases ( $n = 9$ ) it was over 35%. In contrast, outbreaks in which only potentially pathogenic *E. coli* was isolated ( $n = 12$ ) morbidity was lower (5–60%) and mortality was always under 30%. Group C rotavirus was detected in a flock in which there was no enteric disease. Morbidity close to 25%, and no mortality, were observed in both outbreaks of diarrhoea in which group A and group B rotaviruses were detected, but while the former was not apparently the cause of diarrhoea, group B rotavirus infection was clearly associated with the diarrhoea and caused severe growth retardation [13, 18].

None of the outbreaks associated to *Cl. perfringens* infection had a morbidity over 10% but mortality in affected animals approached 100% in all three cases. Their clinical characteristics were different from most of the outbreaks, with diarrhoea being an inconsistent

Table 3. Prevalence of enteropathogens in lambs and goat kids of different ages

|                           |           | Number positive animals/number animals tested (%) |              |              |            |              |
|---------------------------|-----------|---|--------------|--------------|------------|--------------|
|                           |           | 0–5 days  | 6–10 days    | 11–15 days   | 16–45 days | Unknown      |
| Group A rotavirus         | Lambs     | 1/92 (1.1)  | 2/60 (3.3)   | 1/29 (3.4)   | 0/23 (0)   | 0/26 (0)     |
| Group B rotavirus         | Goat kids | 6/58 (10.3)                                       | 0/46 (0)     | 0/20 (0)     | 0/8 (0)    | 0/3 (0)      |
| Group C rotavirus         | Goat kids | 4/58 (6.8)  | 0/46 (0)     | 0/20 (0)     | 0/8 (0)    | 0/3 (0)      |
| Fimbriated <i>E. coli</i> | Lambs     | 28/86 (32.5)                                      | 9/46 (19.6)  | 10/29 (34.5) | 4/20 (20)  | 9/26 (34.6)  |
|                           | Goat kids | 5/20 (25)   | 1/7 (14.3)   | 1/10 (10)    | 1/3 (33.3) | 1/3 (33.3)   |
| VT+ <i>E. coli</i>        | Lambs     | 7/86 (8.1)  | 0.46 (0)     | 2/29 (6.8)   | 1/20 (5)   | 0/26 (0)     |
| <i>C. parvum</i>          | Lambs     | 30/86 (34.9)                                      | 41/60 (68.3) | 0/25 (0)     | 1/20 (0)   | 10/26 (38.5) |
|                           | Goat kids | 7/27 (25.9)                                       | 2/31 (6.4)   | 6/14 (42.8)  | 0.4 (0)    | 0/3 (0)      |
| <i>Cl. perfringens</i>    | Goat kids | 3/20 (15)   | 0/7 (0)      | 1/10 (10)    | 0/3 (0)    | 0.3 (0)      |

sign. Sudden deaths occurred in two outbreaks while prostration and, in some animals diarrhoea that lasted 5–7 days, were the most common signs observed in the other.

The only case from which *Salmonella* sp. was isolated also differed from the rest in its clinical manifestations. Fifteen to 20-days-old goat kids suffered a very watery diarrhoea with high fever, morbidity was 90% and mortality 40%. The three animals necropsied showed haemorrhagic lesions in the lungs and intestine.

## DISCUSSION

*C. parvum* was the most frequently detected agent both in diarrhoeic lambs (45%) and goat kids (42%). The high prevalence of infection, the strong association between infection and diarrhoea and the higher morbidity and mortality rates that were observed in those outbreaks in which it was detected as a single pathogen, suggest that *C. parvum* was the most frequent cause of diarrhoea and one of the most pathogenic of the agents studied. Furthermore, the within-flock prevalence of *C. parvum* infection was also the highest. Most authors have found *C. parvum* to be a major cause of diarrhoea in both species, also, it has been much less frequently detected in non-diarrhoeic animals [3–6, 8]. The prevalences of *C. parvum* infection found in this study (38% in lambs and 19% in goat kids) were similar to those reported by others [3, 4], but lower than that detected in goat kids in France [5] and lambs and goats in Spain [19]. *C. parvum* prevalence may have been underestimated in this study since the oocysts were not concentrated. Both the within-flock prevalence (mean = 53%) and

the age-related prevalence (5–15 days) observed are in agreement with previous reports [3, 5, 6, 20, 21].

*E. coli* with at least one virulence factor was frequently isolated from diarrhoeic lambs (30%) and goat kids (22%). Fimbriae were the most frequent virulence factor with fimbriated *E. coli* being isolated from 26% and 22% of the diarrhoeic lambs and goat kids. These prevalences are higher than that described by most authors [2, 3, 6, 22], although in the majority of these studies only the presence of F5 was investigated. There is little information on the production of ST I by caprine and ovine strains. Cid and colleagues [22] also found a low prevalence (6.5%) of ST I+ *E. coli* in lambs and goat kids in Central Spain, although none of the 138 strains expressed F5 or F41.

It was surprising that none of the fimbriated strains elaborated enterotoxins, and no fimbriae was detected in the only ST I+ strain. Most enterotoxigenic *E. coli* strains isolated from ruminants are F41+ or F5+ and produce ST I [23, 24]. The reason for this association is that both virulence factors are generally encoded in the same plasmid. It is unlikely that the failure to detect ST I in fimbriated strains was due to a low sensitivity of the test used since it has a similar sensitivity to that of the suckling mouse test [25]. The origin and the pathogenicity of these atypical ETEC is unclear. In contrast to other reports [26, 27], ETEC was detected with similar frequencies in all age groups (0–45 days) and no association between infection and diarrhoea was found. Thus, it seems possible that these strains could act as a reservoir of virulence factors more than as pathogens.

VTEC have been previously detected in lamb and goat kid faeces [8, 9, 28], although there is little information on their prevalence. Adesiyun and colleagues [8] isolated VTEC in a 17% of the diarrhoeic

lambs, a higher proportion than the 5.3% found in this study. These authors also failed to demonstrate an association between infection and diarrhoea. In one outbreak only, VTEC was isolated from 57% of the animals, and this could indicate an association to diarrhoea in that instance. The pathogenicity of VTEC in ruminants has not been elucidated although they have been isolated from calves with haemorrhagic colitis [29]. This group of pathogenic *E. coli* is known to cause haemorrhagic colitis and haemolytic uraemic syndrome in humans. Cattle are thought to act as a reservoir for the infection and its presence in sheep suggests this species may also be a reservoir. The absence of LT I and CNF in the strains isolated is not surprising since LT I is considered atypical in ruminant strains and CNF<sup>+</sup> *E. coli* has been detected with low prevalences in diarrhoeic lambs [23, 24, 30]. Three of the strains isolated from lambs produced a toxin that caused a cytotoxic effect on Vero cells similar to that of LT I but it was not identified by the serological tests used and further studies are required to characterize this toxin.

Rotavirus were detected in 2.1% of diarrhoeic lambs and 6.5% of the outbreaks studied in this species. Their prevalence was lower than that reported by others, namely 20–60% in diarrhoeic lambs [2, 4, 31] and 30–60% in outbreaks of diarrhoea [4, 7, 32]. These data contrast with our previous observation of a high prevalence of antirotavirus antibodies in sheep sera in Spain [33]. A good lactogenic immunity transferred to the lambs in a population in which dams have high serum antibody titers, probably derived from transient asymptomatic infections, could have resulted in a reduction in the frequency of rotavirus-induced diarrhoea. A reduction in the prevalence of rotavirus infection in pigs was observed in Austria between 1983 and 1988 [34] and this was attributed to an effective transfer of lactogenic immunity by the sows, since most adults had serum antirotavirus antibodies. A similar change in the prevalence of group A rotavirus infection in lambs was observed in Southern England between 1980 and 1984 [35, 36]. It has been shown that lambs are susceptible to diarrhoea induced by rotavirus for a shorter period than calves [37, 38] and this could have contributed to make lactogenic immunity more effective. The prevalence of group A rotavirus infection in goats was higher (18 animals from 1 out of 14 outbreaks of diarrhoea) than in sheep but also lower than that reported by others [5, 6, 39]. Both group B and C rotaviruses were detected in two different goat

herds and this is the first report of the presence of group C rotavirus infection in goats. While an association between infection and diarrhoea could not be demonstrated in lambs or goat kids for group A, and group C rotaviruses were detected only in non-diarrhoeic animals, a very significant association was found between infection and diarrhoea for group B rotaviruses. Whether or not there exist differences in the pathogenicity among rotavirus groups, or the differences observed are due to the influence of external factors needs to be clarified.

Other enteropathogens such as picobirnaviruses, *Cl. perfringens* and *Salmonella* spp seem to have little or no role in diarrhoea in lambs. Picobirnaviruses have been isolated from cattle, horses and pigs [40, 41], but their role in the pathogenicity of diarrhoea is unknown. In contrast, *Cl. perfringens* may have a more important role in diarrhoea in goats, being detected as a single agent in a 11% of the diarrhoeic goat kids and 20% of the outbreaks. This difference may be due to the more extended practice of vaccination against *Cl. perfringens* enterotoxemia in sheep in North West Spain. The prevalence of this agent could be underestimated since only outbreaks in which diarrhoea was present were included in this study, and diarrhoea can be absent in *Cl. perfringens* infection. *S. arizonae* was isolated from a single outbreak of diarrhoea in goat kids. Its pathological significance is difficult to assess since *C. parvum* was also detected in this outbreak. However, the clinical characteristics corresponded to those described for salmonellosis in young ruminants. None of the samples investigated for the presence of bovine coronaviruses gave a positive result, which suggests either a very low prevalence of infection in both species or that ovine and caprine coronaviruses are not antigenically related to bovine coronavirus. Coronaviruses have been detected on several occasions in diarrhoeic lambs and goat kids [3, 7] but their antigenic composition and their pathogenic significance have not been studied.

Although mixed infections were found both in outbreaks and in individual animals, an association with diarrhoea could not be demonstrated. Other authors have detected mixed infections and in no case did they observe a worsening of the clinical signs or a higher frequency of diarrhoea in animals suffering mixed infections [3, 4, 6]. Experimental infections with ETEC, rotavirus and *C. parvum* [38] suggest little synergism between these enteropathogens in sheep and goats.

No enteropathogens were detected in four and three outbreaks of diarrhoea in lambs and goat kids, respectively. It is possible that other pathogens, not included in this study, were present. In fact, although not investigated routinely, two of the above outbreaks in lambs were associated to septicemic *E. coli* infection. *Eimeria* spp were detected in lambs older than 25 days in one outbreak of diarrhoea together with *E. coli* and in one outbreak alone. Also, the number of samples analysed per outbreak in the cases in which no enteropathogens were detected was low (< 4), which emphasises the importance of examining a large number of samples since many of the agents are often detected in less than half of the samples.

*C. parvum* was the most frequent enteropathogen associated with diarrhoea in lambs and goat kids in North West Spain and consequently its presence should be sought by diagnostic laboratories. Also, farmers should be encouraged to adopt hygienic measures to control this pathogen. Samples should be examined for ETEC infections, but both the presence of fimbria and enterotoxins should be investigated. Diagnostic tests for rotaviruses should be capable of detecting atypical as well as typical rotaviruses, while the investigation of *Salmonella* spp and *Cl. perfringens* may only be necessary if their presence is suspected clinically. Although not included routinely in the diagnostic procedures the possible role of other agents such as attaching-effacing *E. coli*, VTEC and septicemic *E. coli* or *Eimeria* spp in animals older than 25 days should be taken into consideration. The examination of a large number of samples per outbreak, both from diarrhoeic and non-diarrhoeic animals may assist in assessing the pathogenic role of the enteropathogens detected.

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