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Short communication

Relationship between the treatment and the evolution of the clinical course in scouring Merino lambs from "La Serena" (Southwest Spain)

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Abstract

This work investigated the link between the type of treatment and the clinical evolution of lambs suffering from diarrhoea attributed to non-enterotoxigenic *Escherichia coli*. Two hundred and forty scouring lambs, and 25 healthy lambs selected as control, were used in this trial. The faecal samples from the scouring lambs were positive to non-enterotoxigenic *E. coli*. All the scouring lambs received supportive care and they were randomly allotted to two groups of 120 animals (treated group and untreated group). The lambs in the treated group were given two daily doses of 20 mg/kg live weight spectinomycin for 3 days, while the other group of lambs (untreated group) did not receive any antibiotic. Serum endotoxin was higher in the treated lambs. The combined infection of *E. coli* + *Proteus mirabilis* was the most frequent microbiological result in the deceased treated lambs, while the only enteric pathogen isolated in the untreated lambs submitted to necroscopy was *E. coli*. The pathological findings most commonly recorded in the untreated lambs were suggestive of a generalized inflammatory process attributed to colibacilosis, while the lesions in the treated lambs might correspond to an enterotoxoemic process. The overproduction of *P. mirabilis* might be consequence of the antibiotic treatment and it would be the most probable cause of the endotoxemia, the high mortality rate and the pathological findings in the treated lambs. Therefore, a supportive care without antibiotics does not lead to a poorer chance of survival in lambs with diarrhoea attributed to non-enterotoxigenic *E. coli*.

Keywords: Lamb; Diarrhoea; Spectinomycin; Endotoxemia; *Proteus*; *E. coli*

1. Introduction

Diarrhoea in lambs is a complex, multi-factorial disease involving animal, environment, nutrition and infectious agents. Several factors are able to predispose to diarrhoea. Flock size, type of facilities, type of breeding, lambing percentage, isolation of *Campylobacter jejuni*, *Rotavirus* spp., *Coronavirus* spp. and *Salmonella* spp. seem to be poorly correlated to lamb mortality. On the contrary, bad cleaning of the lambing areas, continuous lambing periods, accumulation of lambs in the pens, high content of fat, protein and lactose in milk, low serum gamma globulin and total protein in lambs and ewes, and *Cryptosporidium* spp. infection are strongly

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linked to lamb mortality (Muñoz et al., 1996; Andrés et al., 2007).

It has been stated that some factors could affect the survival chance of the animals suffering from this disorder. The effect of endotoxemia is particularly important, as a relationship has been found between the presence of endotoxin in blood and the unfavourable prognosis of the scouring syndrome (Jiménez et al., 2007).

This piece of research investigated the possible relationship among the type of treatment, the evolution of the clinical course and the severity of the pathological findings in lambs severely affected by diarrhoea.

2. Materials and methods

Eighteen extensive sheep farms with previous history of high lamb losses attributed to scouring were randomly selected for this experiment. At the time of the trial all the flocks presented natural outbreaks of diarrhoea affecting lambs in the first 2 weeks of life, with a prevalence of 20–80%. The farms were located in the same geographical area (La Serena, Southwest Spain) and they were managed under similar health, nutrition and husbandry practices.

On each of the 18 farms, lambs were examined to identify "scouring lambs" with active diarrhoea, fever, tachypnea, dullness and a dehydration of 5–10%. The number of lambs selected in each farm was proportional to the prevalence of the scouring syndrome in the flock, with a total of 240 lambs with diarrhoea included in the study.

At the time of the first physical examination samples of faeces were taken from the rectum of the lambs using sterile culture swabs (eurotubo®, IASA, Barcelona, Spain). The scouring lambs received 400 mL of an intravenous Ringer lactate solution (Solución Ringer Lactato®, Braun, Barcelona, Spain) initially, and then they were rehydrated as many times as necessary. After 24 h, once the etiological diagnosis was known, half of the patients were treated with two daily doses of 20 mg/kg live weight spectinomycin for 3 days (Spectamporcelet®, Ceva, Barcelona, Spain) and an oral dose of 0.07 mg/kg live weight halofuginone (Halocur®, Intervet, Spain) once a day for 3 days, while the rest of the animals only received halofuginone.

All the animals were under veterinary supervision. Experienced teams of observers monitored lambs at regular intervals to assess the outcome of treatment. During this period the lambs with unfavourable evolution were submitted to a final clinical examination and 2.5 mL blood were withdrawn from the jugular vein of each lamb into sterile clotting, EDTA and 3.2% trisodium citrate tubes (eurotubo®, IASA, Spain). At the same time, a certain number of healthy lambs in each farm, 25 in total (control group), were selected and sampled for comparison of the levels of endotoxin and fibrinogen in blood. Then, the deceased lambs were submitted to necroscopy and further pathological and microbiological studies.

2.1. Blood analysis

Blood samples were processed for serum and plasma separation. Serum samples were assayed for endotoxin with the chromogenic LAL test according to the manufacturer's instructions (chromogenic LAL lysate test QCL 1000®, Cambrex Iberia Products, Barcelona, Spain) by photometry (Shimazdu UV 160®, Pacisa, Barcelona, Spain). Plasma fibrinogen was measured with a fibrinogen assay kit (Thrombin 200, Pacific Hemostasis, Huntersville, USA) in an automatic coagulometer (CLOT 1, Pacisa, Barcelona, Spain).

2.2. Pathological examination

The lambs were examined post-mortem for gross evidence of disease and samples were taken from central nervous system (CNS), lungs, liver, spleen, kidneys, abomasum, jejunum and mesenteric lymph glands for pathological and microbiological analysis. Tissues for pathological study were fixed in 4% buffered formaldehyde solution by standard paraffin-embedding methods. Five micrometers thick sections were cut and treated with haematoxylin and eosin.

2.3. Detection of enteric pathogens

The infectious agents involved in scouring were investigated by culture in appropriate media, immune assay and PCR. Enteric bacteria were cultured on Agar MacConkey® (Oxoid, Madrid, Spain) and Agar XLT4® (Merck, Barcelona, Spain). Blood Agar was used for the anaerobiotic culture of *Clostridium* spp. (Blood Agar®, Oxoid, Spain). Brucella Agar was used for *Campylobacter* spp. (Modified Brucella Agar®, Oxoid, Spain). The cultures were carried out according to the procedures of Carter and Chengappa (1990). Commercial immunochromatographic faecal antigen detection tests, validated for use in ruminants, were performed as recommended by the manufacturer. These tests were used to detect antigens of *Rotavirus* spp. (Rota Vet Uni-Strip®, Coris Bioconcept, Brussels, Belgium), *Coronavirus* spp. (Corona Vet Uni-Strip®, Coris Bioconcept, Belgium), *Crytosporidium* spp. (Crytpo Vet Uni-Strip®, Coris Bioconcept, Belgium) and enterotoxigenic *Escherichia coli* K99 (K99 Vet Uni-Strip®, Coris Bioconcept, Belgium) in faeces. A PCR technique validated for use in sheep was carried out according to the work of Rey et al. (2003). This method was used to identify virulent *E. coli* genes (primers and PCR mixture, Amersham Biosciences, Barcelona, Spain).

2.4. Statistical analysis

After checking the normality of the distribution of data, the differences between the concentrations of endotoxin and fibrinogen in the blood of the lambs in the control, the treated and the untreated groups were tested for statistical significance by using ANOVA. Contingency tables and chi-squared test were employed to evaluate the statistical significance of any differences for case fatality rates, finding of enteric pathogens

Table 1
Number of animals in the experiment, case fatality rate, serum endotoxin and plasma fibrinogen levels (mean ± standard error) in the lambs from the treated and untreated groups

Number of lambs scouring ($n = 240$)	Control lambs ($n = 25$)	Treated lambs ($n = 120$)	Untreated lambs ($n = 120$)
Serum endotoxin level (ng/mL)	0.14 ± 0.01 a	0.48 ± 0.01 b	0.31 ± 0.12 c
Plasma fibrinogen level (mg/dL)	114 ± 15 a,*	801 ± 92 b	723 ± 65 b
Case fatality rate (%)		68.3 a	17.5 b

a, b and c mean with different letters in each row are significantly different ($P < 0.05$, $^*P < 0.001$).

Table 2
Occurrence (%) of the main pathological findings in the deceased lambs from the treated and untreated groups and *E. coli* + *Proteus* spp. detection

	Treated lambs $n = 82$	Untreated lambs $n = 21$	χ^2	P
Intestine				
Catarrhal gastroenteritis	98.1	95.2	1.1008	0.2939
Enlargement and hyperplasia of lymphoid organs	97.6	90.5	2.2482	0.1338
CNS				
Congestion and neuronal degeneration	61.0	33.3	5.1687	0.0230
Non-purulent meningoencephalitis	37.8	66.6	5.6608	0.0173
Lungs				
Congestion and emphysema	58.5	28.6	6.0189	0.0142
Interstitial pneumonia	45.1	76.2	6.4605	0.0110
Liver				
Congestion and degenerative changes in hepatocytes	64.6	38.1	4.8759	0.0272
Perivascular hepatitis	31.7	57.1	4.6457	0.0311
Kidneys				
Tubulonephrosis	57.3	33.3	3.8558	0.0496
Interstitial nephritis	41.5	66.6	4.2676	0.0385
E. coli + *Proteus*	62.2	38.1	3.9682	0.0464

and occurrence of the main pathological findings, between the treated and untreated deceased lambs. The statistical package G-Stat (Letón and Marino, 2002) was used.

3. Results

Faecal cultures from all the scouring lambs in all the farms ($n = 240$) were positive for *E. coli* and most of them for *Cryptosporidium* spp. However, PCR assays yielded negative results for enterotoxigenic or verotoxigenic *E. coli* strains and for *Rotavirus*, *Coronavirus* and *Salmonella*. *C. jejuni* was isolated only in one farm.

The results of case fatality rate (%), serum endotoxin level (ng/ml) and concentration of fibrinogen (mg/dl) in plasma in the different groups of lambs are displayed in Table 1. The first two parameters were significantly higher ($P < 0.05$) in the treated lambs. However, no difference was found for plasma fibrinogen level between the treated and the untreated group.

The occurrence of the main pathological findings in the treated and untreated deceased lambs is shown in Table 2. The patients in both groups presented some common pathological findings, as catarrhal gastroenteritis and the typical reactivity pictures in the lymphoid organs, with enlargement and hyperplasia. Nevertheless, certain lesions in other systems appeared with different incidence in the two groups.

The results of the microbiological study from the samples obtained at the necroscopy revealed the presence of *E. coli*, *Proteus* spp. and, scarcely, *Cryptosporidium* spp. The frequency of mixed infections of *E. coli* and *Proteus* spp. is indicated in Table 2.

4. Discussion

In this trial the main enteric pathogens in the scouring lambs were *Cryptosporidium* spp. and *E. coli*. The role of these agents in the production of diarrhoea in young small ruminants has been stated in this region (Muñoz et al., 1996) and worldwide (Matthews, 1999).

The concentration of endotoxin in the lambs in the two groups of scouring lambs was significantly higher than in the control group (Table 1). The level of endotoxin in the serum of the control lambs was similar to

that reported for healthy horses (Barton and Collatos, 1999), human blood donors (Nadhazi et al., 2002) and lambs (Jiménez et al., 2007). The lambs in the treated and untreated groups presented levels of serum endotoxin comparable to those found in horses affected by colic (Barton and Collatos, 1999) and in human patients suffering from septic shock (Danner et al., 1991).

Plasma fibrinogen is an acute phase hepatic protein. It is an indicator of occult inflammatory disorder in ruminants; the greater the magnitude of its increase, the greater the magnitude of inflammation (Kramer, 2000). The concentrations of fibrinogen in the treated and the untreated lambs were significantly higher than in the control lambs (Table 1). This is indicative of a severe inflammatory process.

Many lambs in the two groups presented catarrhal gastroenteritis and enlargement of the lymphoid organs, as reported in lambs suffering from scouring syndromes related to the same aetiology (Barker and Van Dreumel, 1985).

The pathological findings recorded with a significantly higher frequency in the untreated lambs (Table 2) were suggestive of a generalized inflammatory process attributed to colibacilosis, which is characterized by meningoencephalitis, interstitial pneumonia, perivascular hepatitis and interstitial nephritis (Barker and Van Dreumel, 1985).

The pathological findings registered more commonly in the treated lambs (Table 2) might correspond to an enterotoxemic process. The inflammation of the intestine attributed to *E. coli* would cause an ischemic injury to the digestive mucous barrier. In this instance, large quantities of endotoxin would be absorbed from the intestine, overwhelming the detoxifying ability of the liver and increasing the concentration of endotoxin in the peripheral circulation (King and Gerring, 1988; Haussman et al., 2000). The presence of toxins in the organs would be responsible of the cell degeneration (Haussman et al., 2000), as suggested by the significantly higher concentration of endotoxin in the plasma of the treated lambs (Table 1).

As indicated by the low frequency of isolation of *Cryptosporidium* spp. in the lambs submitted to necroscopy, the treatment with halofuginone was highly effective, as it has been reported in calves (Lefay et al., 2001). The combined infection of *E. coli* + *P. mirabilis* had a higher incidence in the treated lambs (Table 2), while the only enteric pathogen isolated in the untreated lambs was *E. coli*. This finding might be associated to the effect of the antibiotic treatment. Spectinomycin is a bacteriostatic antibiotic that inhibits protein synthesis in susceptible bacteria by binding to the 30S ribosomal unit (Plumb, 1995). The efficacy of antibiotic treatment against *E. coli* (Hodgson et al., 1999) and the resistance of *P. mirabilis* against antibiotics in several circumstances (Dance et al., 1987) have been reported. This could indicate that the treatment with spectinomycin could have limited the growing of *E. coli* in the patients from the treated group and it would have permitted the overproduction of *P. mirabilis*.

P. mirabilis is able to produce endotoxin that can impair diverse biological functions through several mechanisms, as the alteration of platelet activity (Saluk-Juszczak et al., 2007). In the treated lambs the concentration of endotoxin in serum was significantly higher than in the untreated group (Table 1). This concentration was around 0.50 ng/mL, the point beyond survival chance is null (Jiménez et al., 2007); while untreated lambs showed results below this limit. This could explain the significant difference in the mortality rate between the two groups (Table 1). It could support as well the pathological findings of the spectinomycin-treated lambs lost by diarrhoea.

The proliferation of *P. mirabilis*, the elevated endotoxin concentration, the higher mortality rate and the pathological findings in the treated group appeared to be related to the antibiotic treatment. Therefore, it might be considered that, under the management and husbandry conditions of this trial, a supportive care without antibiotics would not lead to a poorer chance of survival in lambs with diarrhoea attributed to non-enterotoxigenic *E. coli*. Another important benefit related to the reduction in the use of antibiotics would be the decrease of residues in meat.

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