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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)

L. Gómez [a], S. Andrés [a], J. Sánchez [a], J.M. Alonso [b], J. Rey [b], F. López [c], A. Jiménez [a,*]

[a] *Department of Animal Medicine, Faculty of Veterinary Science, University of Extremadura, Campus Universitario, 10071 Cáceres, Spain*
[b] *Department of Animal Health, Faculty of Veterinary Science, University of Extremadura, Campus Universitario, 10071 Cáceres, Spain*
[c] *Department of Technological Development, Junta de Extremadura, Enrique Díaz Canedo s/n, 06800 Mérida, 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

* Corresponding author. Tel.: +34 927257164; fax: +34 927257110.
E-mail address: ajredon@unex.es (A. Jiménez).

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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