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ScienceDirect
Small Ruminant Research 70 (2007) 272–275
Small Ruminant Research
www.elsevier.com/locate/smallrumres

Technical note

Evaluation of some etiological factors predisposing to diarrhoea in lambs in "La Serena" (Southwest Spain)

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Received 27 September 2005; received in revised form 9 March 2006; accepted 10 April 2006
Available online 24 May 2006

Abstract

This study compares some etiological factors involved in the naturally occurrence of lamb scours in 18 Merino sheep farms randomly selected in the area of "La Serena" (Southwest Spain).

A lack of influence of some variables (flock size, type of facilities, type of breeding, lambing percentage, isolation of *Campylobacter jejuni*, *Rotavirus* spp., *Coronavirus* spp. and *Salmonella* spp.) on lamb mortality rate was detected. The opposite was true for cleaning of the lambing areas, organization of lambing periods, accumulation of lambs in the pens, high content of fat, protein and lactose in milk and low serum gamma globulin and total protein in lambs and ewes. *Cryptosporidium* spp. and *Escherichia coli* isolation showed a trend to be linked to the presence of diarrhoea. A positive correlation was found between serum total protein in ewes and serum gamma globulin in lambs. Thus, the measure of serum total protein in ewes before lambing would be a valuable indicator of the risk of lamb scouring and it would be useful to establish an adequate programme to prevent this condition in the farms of this geographical area.
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Keywords: Lamb; Diarrhoea; Predisposing factors; Milk composition; Gamma globulin

1. Introduction

"La Serena" is one part of Extremadura (Southwest Spain) with more than one million sheep. In this area, lamb scouring is of paramount economical importance. Only in the three most prominent farm associations losses are estimated in approximately 314 million Euro per year, and they are attributed to the high mortality rate, the reduced weight gain and the cost of the treatments (Agriculture Research Service, personal communication).

The etiology of this syndrome is multiple, including infectious agents as *Escherichia coli* and *Cryptosporidium* spp. (Muñoz et al., 1996), management and reproductive factors (flock size, lambing percentage, accumulation of lambs in the pens, cleaning of lambing areas) and the nutritional and immune status of ewes and lambs.

This experiment is part of a wide 3 year-long study which evaluates the factors predisposing to lamb scouring in this region; and it was undertaken to provide a correct guide to deal with this problem for farmers and veterinary practitioners.

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doi:10.1016/j.smallrumres.2006.04.004

2. Materials and methods

Eighteen Merino sheep farms with previous history of lamb diarrhoea were randomly selected. Eight of them presented during the study mortality rates in lambs and attributed to scouring higher than 15% (high mortality), while the other ten showed lower mortality rates (low mortality). This ratio was verified holding contacts with the farms to quantify the losses.

Flocks were managed under semi-extensive system, with the same health and management practices. Nutrition was based upon grazing and supplementation in the seasons with poor forage production (summer and the coldest months of winter) and in the lactating period.

Farms were inspected and information was collected about flock size (below average level of 700 head, above average), type of facilities (old, new), type of breeding (natural, synchronised), lambing percentage (below average point of 70%, above average), organization of lambing periods (continuous, discontinuous), cleaning of lambing areas (bad, good) and accumulation of lambs in the pens (>2, <2 lambs/m^2). Milk and blood samples were taken from ewes, and blood samples from their lambs in a number proportional to flock size (<400 head = 10 ewes and their lambs, 400–800 head = 15, >800 head = 20). Lambs were 15 days old and did not show clinical signs of diarrhoea or dehydration in that moment. All the ewes were homogeneous in age and body condition. Clinical reports of lambs from the selected farms, referred to the Diagnostic Service of the Veterinary Teaching Hospital of Extremadura University during the last months were used to investigate the infectious agents involved.

2.1. Blood samples

Samples were taken by jugular venipuncture and put into clotting activating vacuum tubes. Serum total protein was measured by spectrophotometry (Shimazdu UV 160 A®, Total Protein Liquicolor® Human) and protein fractions were separated by cellulose acetate electrophoresis (Pherotank®, Gernon), stained with Ponceau Red and read by means of a densitometer (Scanion LRA®, RAL).

2.2. Milk samples

Milk samples were obtained manually and then refrigerated until analysis. Fat, protein and lactose contents were measured by near infra-red techniques (Milkoscan Electric®, Foss).

2.3. Finding of enteropathogens

Culture in specific media, immune assay and PCR were employed. Enteric bacteria: Agar MacConkey ® (Oxoid) and Agar XLT4® (Merck); *Clostridium* spp.: Blood Agar® (Oxoid); *Campylobacter*: Modified Brucella Agar® (Oxoid); *Rotavirus* spp.: immune chromatographic test Rota Vet® (Fastia Diagnostics); *Coronavirus* spp.: immune chromatographic test Corona Vet® (Fastia Diagnostics); *Crytosporidium* spp.: immune chromatographic test Crypto Vet® (Fastia Diagnostics); enterotoxigenic *E. coli:* immune chromatographic test COLI K99 Vet® (Fastia Diagnostics); *E. coli* specific genes for virulence factors: primers and PCR mixture (Amersham Biosciences).

2.4. Statistical analyses

The dependent variable used throughout this study was the number of farms with high and low lamb mortality rate. The independent variables were flock size, type of facilities, reproductive management, organization of lambing periods, lambing percentage, cleaning of lambing areas, accumulation of lambs in the pens, milk fat, milk protein, milk lactose, serum gamma globulin in lambs, serum total protein in lambs, serum gamma globulin in ewes, serum total protein in ewes, isolation of *Cryptosporidium* spp., *E. coli*, *Campylobacter jejuni*, *Rotavirus* spp., *Coronavirus* spp and *Salmonella* spp.

The relationship between the dependent and the independent variables was studied by calculating Chi-square (χ^2) value and odds ratio (OR). Correlation tests were performed between the individual levels of serum globulin and serum total protein in lambs, serum gamma globulin in lambs and serum gamma globulin in ewes, and between serum gamma globulin in lambs and serum total protein in ewes. The statistical package G-Stat 2.0 was used (Letón and Marino, 2002).

3. Results

Among the variables contained in the information collected in the visits to the farms (flock size, type of facilities, type of breeding, lambing percentage, organization of lambing periods, cleaning of lambing areas and accumulation of lambs in the pens) only the three later were significantly related to lamb mortality.

The number of farms with high and low lamb mortality according to the magnitude (under or above average values) of milk and blood variables, together with the values for OR, χ^2 and statistical significance appear in

Table 1
Statistical results from the comparison between the number of farms with high and low lamb mortality and the categories (under or above average values) of some variables involved in outbreaks of lamb scouring in the region of "La Serena" (Southwest Spain)

Variable	Category	Number of farms		Odds ratio	χ^2	P
		High mortality	Low mortality			
Milk	<8.16	2	8			
Fat (%)	≥8.16	6	2	12.0000	5.4450	0.0196
Milk	<6.19	3	9			
Protein (%)	≥6.19	5	1	15.000	5.5125	0.0189
Milk	<4.90	3	9			
Lactose (%)	≥4.90	5	1	15.000	5.5125	0.0189
Serum	<1.30	7	4			
Gamma globulin in lambs (g/dL)	≥1.30	1	6	10.500	4.2195	0.0400
Serum	<5.80	7	4			
Total protein in lambs (g/dL)	≥5.80	1	6	10.500	4.2195	0.0400
Serum	<1.30	5	1			
Gamma globulin in ewes(g/dL)	≥1.30	3	9	15.000	5.5125	0.0189
Serum	<5.94	6	1			
Total protein in ewes (g/dL)	≥5.94	2	9	27.000	7.9013	0.0049

Table 1. Significant results were recorded for all variables.

Positive correlations were found between the individual values of gamma globulin and serum total protein in lambs ($R = 0.728$, $P = 0.012$), between the individual levels of gamma globulin in ewes and their lambs ($R = 0.670$, $P = 0.033$) and between serum gamma globulin in lambs and serum total protein in ewes ($R = 0.632$, $P = 0.027$).

The most relevant microbiological findings obtained from the clinical reports of previous scouring occurrences in the same farms were: *E. coli* isolated in all the farms, *Cryptosporidium* spp. frequently isolated, *C. jejuni* only isolated in one farm and *Rotavirus* spp., *Coronavirus* spp. and *Salmonella* spp. not detected.

4. Discussion

The lamb mortality rate was independent of the flock size (below or above 700 head), in contrast to the findings of Causape et al. (2002), who recorded a higher risk of infection in larger flocks. Neither the type of facilities appeared to have influence on the onset of diarrhoea, which might be explained by the fact that cleaning and health practices do not need to be related to how the facilities are kept. The same lack of effect was observed for the type of breeding and the lambing percentage. Synchronization rises lambing percentage, but it does not imply more concentration of lambs in the pens when the facilities are adequate.

The organization of lambing periods, the cleaning of lambing areas and the accumulation of lambs in the pens seem to be predisposing causes to neonatal diarrhoea in this area. Continuous breeding in a flock means lambing during all the year, and the unbroken permanence of lambs in the pens, hampering cleaning and resulting in the coexistence of lambs of different ages. This permits the accumulation of pathogens and a progressive recharge of the faecal-oral cycle (Coop and Wrigth, 2000). The poor hygiene, together with the accumulation of lambs in the pens (more than 2 lambs/m^2), contributes to the contamination of the area and the spreading of the disease (Radostits et al., 1999; Causape et al., 2002).

The results obtained in milk analyses were similar to those reported by Izquierdo et al. (2003) in Merino sheep and in our same field conditions. A positive relationship was recorded between milk composition and the presence of diarrhoea (Table 1). When large quantities of very rich milk are ingested, the digestive ability of the abomasum is exceeded, and milk clotting is impaired. Partially digested milk proceeds along the small bowel, resulting in high concentration of nutrients, specially lactose, in the gut lumen, withdrawing water from interstitial space and favouring the onset of diarrhoea (Radostits et al., 1999). In addition, due to the climatic conditions of the area, sheep receive more supplements during the lambing period of autumn-winter and they presumably produce richer milk in this season (Izquierdo et al., 2003).

One of the most important etiological factors of the diarrhoeic syndrome is lamb immune deficiency. Jiménez et al. (1993), under similar field conditions in other areas of Extremadura, obtained significant differences for gamma globulin between healthy and scouring lambs. In the present experiment a direct relationship between serum gamma globulin in lambs and mortality was recorded (Table 1). Average gamma globulin levels

were 1.3 g/dL. Therefore, in this area and under these husbandry conditions attention should be paid to flocks with average serum gamma globulin in lambs below this point.

An association of the same magnitude and direction was found between serum total protein in lambs and lamb mortality (Table 1). In calves, serum protein values lower than 5.0 g/dL are related to the predisposition to scouring (McGuire and Adams, 1982). In our experiment this limit would be 5.8 g/dL, the average serum protein value. This influence of serum total protein on lamb mortality rate is supported by the positive correlation found between the individual values of serum total protein and gamma globulin in lambs. This would be very interesting in this area, from a practical point of view; as it would permit to employ this easy technique (clinical rephractometry) instead of more complicated ones such as electrophoresis and laser nephelometry for measuring the risk of lamb scouring.

Low immunity in lambs may be caused by a lower level of immune globulin in the dam or a failure of immune passive transference (FPT) (Tizard, 1996; Rogers, 2000). In this experiment, subnormal gamma globulin levels were obtained in ewes (average = 1.3 g/dL). This low value suggested that the poor immune status observed in the lambs of the experiment might be linked to the low immune levels of their dams and not to FPT. This was confirmed by the positive correlation between the individual levels of gamma globulin in ewes and their lambs. This fact explained that below 1.3 g/dL more farms showed high mortality (Table 1). In our case, this limit would be a good predictor of the probability of lamb scouring episodes in a certain flock.

Average values of serum total proteins in ewes were slightly below the range for a normal adult sheep (Kaneko et al., 1997). This variable had a significant relationship with the number of high and low mortality farms (Table 1) and its origin lies in the positive correlation detected between serum gamma globulin in lambs and serum total protein in ewes. Consequently, the measure of serum total protein in ewes before lambing would be a valuable indicator of the risk of lamb scouring and it would be useful to establish an adequate programme to prevent this condition in the farms of this geographical area.

Our microbiological findings were in accordance with others previously reported by other workers in the same area (Muñoz et al., 1996), except for *E. coli*, detected in all the farms of our experiment. These results suggest the role of *Cryptosporidium* spp. as primary infection and the involvement of *E. coli* in the development of the condition studied, mainly in relation to its ability to cause losses by endotoxemia.

Acknowledgements

This work has been supported by the "Department of Education, Science and Technology, Regional Government" and "European Funds for Regional Development" (2PR03B024). We would like to thank to the "Laboratorio Agroalimentario de Extremadura (Department of Agriculture, Regional Government)" for its assistance in milk analyses.

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