

Prevention of Scours in Neonatal Kids after Oral Administration of an Organic Acid Solution or Antibiotics

S. K. Kritas*, A. R. Burriel¹, A. H. Tzivara, S. C. Kyriakis², H. Karatzias² and J. Vlemmas³

Clinic of Medicine Faculty of Veterinary Medicine, University of Thessaly, Karditsa 43100, Greece

ABSTRACT : The efficacy of an organic acid solution in the prevention of neonatal kid diarrhoea, was examined in this study and was compared with that of different antibiotics. In a goat farm, kids and their mothers were divided in 4 experimental groups. The kids of Group I were not given any treatment serving as negative controls. The kids of Group II were treated orally for 3 consecutive days with a solution of organic acids starting approximately 12 h after their birth. The kids of Groups III and IV were treated once orally with ampicillin and oxytetracycline respectively, approximately 12 h after birth. All groups were compared as regards the occurrence of diarrhoea, its duration, and the rate of mortality. The results have shown that the number of cases and the duration of diarrhoea were significantly reduced in all treated groups, when compared to the control group ($p < 0.05$). However, no or little difference was noted with respect to morbidity, mortality and diarrhoea characteristics when treated groups were compared with each other ($p > 0.05$). Samples of diarrhoeic faeces from kids in the control group resulted in the isolation of K88 and K99 strains of *Escherichia coli*. It was concluded that, early oral administration of organic acids can be effective in the prevention of scours in neonatal kids, possibly caused by enterotoxigenic *E. coli* strains, and to a degree of protection similar to that seen with the used antibiotic schemes. (*Asian-Aust. J. Anim. Sci.* 2002. Vol 15, No. 7 : 1040-1044)

Key Words : Kid, Colibacillosis, Diarrhoea, Prevention

INTRODUCTION

Goat breeding represents an important branch of animal farming for several countries. In most cases, this type of farming is kept under traditional and nomadic conditions, thus animals are subjected to extreme climatic changes depending on the season of the year. Such changes may booster the emergence of severe scours and mortality in neonatal kids caused by enterotoxigenic strains of *Escherichia coli* (ETEC), *Clostridium perfringens* (types A, B and C), rotavirus, *Salmonella* spp or, less frequently, *Cryptosporidium* spp. and *Eimeria* spp. (Merck, 1998 a,b; Radostits et al., 1999; Cooper, 2000; Navarre et al., 2000 and Van Metre et al., 2000).

To prevent diarrhoea in high-risk farms, an established practice is the prophylactic administration of antimicrobials (Radostits et al., 1999). A more environmental-friendly way currently applied in newly-weaned piglets and calves in order to prevent *E. coli* infections, is the administration of organic acids in their water or feed (Fallon and Harte, 1980;

Thomlinson and Lawrence, 1981; Kyriakis et al., 1996a,b; Tsiloyiannis et al., 1998a,b). In pigs, for instance, post weaning diarrhoea syndrome and oedema disease can be effectively prevented by organic acids, sometimes to the same degree as with antibiotics, as can be easily concluded by the improvement in diarrhoea scores, mortality and growth characteristics (Tsiloyiannis et al., 1998a,b). A similar protective effect of these acids has not be ascribed in small ruminants. The purpose of this study was to investigate, under field conditions, the protective effect of organic acids in neonatal kids suffering from ETEC-caused scours and to measure clinically the degree of this protection. Finally, a comparison with empirically used antibiotic prophylactic schemes was attempted.

MATERIALS AND METHODS

Animal herd

This field study was conducted in a dairy goat farm of 1,200 does located on a mountainous chilly area of Central Greece (altitude 800 m). The entire milk production was used exclusively in the farm's cheese making facilities. Does were of native breed (*Capra prisca*) and were living in slatted yards. The 1,200 does were divided into two groups of parturition. One group was set for early parturition taking place around January, and the other for late parturition taking place around March, although small variations had been observed from year to year. Each lactating doe was fed daily 350 g alfalfa hay, 800 g tomato silage, 300 g sugar beet and 500 g concentrated feed (approximately 50% corn, 10% soybean meal, 10% wheat

* Corresponding Author: S. K. Kritas. Tel: +30-441-66003, Fax: +30-441-66055, E-mail: skritas@vet.uth.gr

¹ Laboratory of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Thessaly, Karditsa 43100, Greece.

² Clinic of Productive Animal Medicine, Faculty of Veterinary Medicine, University of Thessaloniki, Thessaloniki 54006, Macedonia, Greece.

³ Laboratory of Pathology, Faculty of Veterinary Medicine, University of Thessaloniki, Thessaloniki 54006, Macedonia, Greece.

bran, 25% barley and 5% vitamins, minerals and trace elements). In addition, does had a daily access to pasture. The herd was free of brucellosis. An antiparasitic programme with the administration of albendazole twice a year (October and May) was applied in the farm. All does were vaccinated before parturition against *C. perfringens* types B, C and D, *C. septicum*, *C. chauvoei*, *C. tetani* and *C. novyi* type B (Heptavac[®], Hoechst). They were vaccinated twice if they were in the group of does in early parturition, and three times when in late parturitions.

At the 1st day of life, all newborn kids were receiving prophylactically 1 ml of Flatulex[®] (Veterin, Greece), a prepartate containing 17 mg vitamin E acetate and 1.67 mg sodium selenite per ml. Feeding of kids was only by natural suckling (no additional artificial feeding was applied in the farm). Kids were weaned at 2 to 2.5 months of age and immediately slaughtered. During the first week after birth both does and kids were housed in a restricted area, not allowed to pasture.

Recent farm history

One of the main problems during the previous years in that farm was the high proportion of severe diarrhoea among neonates, resulting in a mortality rate being close to 70%. This problem was reported to occur during late kidding seasons when weather was cold and wet, in contrast to those seen during early kidding seasons with mortality being less than 10%. Laboratory examinations performed during the last late kidding period in faecal samples from ill kids, have shown the absence of coccidial oocysts, *Cryptosporidium* spp. and enterobacteriaceae, but only *E. coli*. The strains were identified as belonging to F4 (K88) and F5 (K99) antigenic serotypes (data not published).

Field trial

Three hundred pregnant does expected for late parturition were ear tagged and divided into four groups of 75 does each. After kidding, the kids of Group I were left untreated and served as negative controls. The kids of Group II received 5 ml of a 10% aqueous solution of a commercially available mixture of organic acids (Euroacid 50-L, Eurotec, Belgium) for 3 consecutive days, starting approximately 12 h after birth. The mixture contains fumaric, citric, orthophosphoric and malic acids. Only for the purpose of the trial and in order to ensure the proper ingestion of the right quantity, the mixture was administered using a gastric tube (but can be otherwise orally administered by using a syringe or by diluting it accordingly in the milk replacer). The kids of Groups III and IV received a single oral dose containing 20 mg/kg ampicillin trihydrate (Penbritin oral, Smith Kline Beecham-Pfizer) and 25 mg/kg oxytetracycline hydrochloride (Terramycin powder oral sol, Pfizer) respectively, approximately 12 h after birth. The latter antibiotic

treatments are empirically used by farmers of that area for preventive purposes. The eventual number of kids treated in each group were, 110 in Group I, 112 in Group II, 105 in Group III and 109 in Group IV. For the duration of the trial, none of the ill kids in the control group received any treatment.

The clinical signs, the number of kids showing diarrhoea, the duration of diarrhoea (diarrhoea scores), and deaths among the ill animals were daily recorded. Scoring of diarrhoea was based on a) the scouring period of the group for the length of late parturition season, which is the sum of the scouring duration of all kids in the group during this season, b) the scouring period per kid, which is the scouring period of the group divided by the number of all the kids, including the healthy ones, and c) the scouring period per sick kid, which is the scouring period of the group divided by the number of sick kids in the group.

Faecal materials obtained from 10 scouring kids of Group I and three of each of the other groups were submitted for laboratory examination to determine the pathogens involved in the diarrhoea. The faecal samples were examined by a) faecal flotation for the presence of coccidial oocysts, b) modified Ziehl-Neelsen staining for *Cryptosporidium* spp. and c) culturing for bacteriological isolation of enterobacteria. Isolations were attempted on MacConkey and Salmonella-Shigella (SS) agars (Merck KGaA, Germany). The inoculated culture media were incubated in 37°C for 24 h. Colonies having phenotypic differences were subcultured on Columbia blood agar (CBA) if they were observed on MacConkey agar, and on MacConkey if they were observed on SS agar. All apparently pure cultures from MacConkey agar were sampled with a swab and subcultured on CBA. Those identified as haemolytic, gram-negative, oxidase negative rods were further identified to species level using the API 20E kit (BioMerieux, France). Those strains identified by the API 20E as *E. coli* were further serotyped using the Fimbrex K88, Fimbrex K99 and Fimbrex 987P latex agglutination kits, employing monoclonal antibodies against F4 (K88), F5 (K99) and F6 (987P) fimbrial antigens, respectively (Veterinary Laboratory Agency, UK).

Statistical analysis

Statistical analysis has been performed using chi square (Fisher's exact test) to compare morbidity, mortality and case fatality rates, or by one-way analysis of variance (ANOVA) coupled with Duncan's test for comparing means of diarrhoea parameters (SPSS Version 8.0 for Windows, 1997; SPSS Inc., USA).

RESULTS

Clinical observations

In general, the affected animals were showing diarrhoea

starting about two days after birth, severe dehydration, depression, weakening, coma and death within the next 2 days of their life. Stools were watery in consistency and white to yellow in color, not containing any blood. No sudden deaths, neurological symptoms, icterus, or change in urine color were observed in the affected neonates. Post-mortem examination of dead kids has shown inflammation of the mucosa of the small intestine with no other findings (e.g. haemorrhagic enteritis or icterus).

From the 110 kids of the control group, 96 showed clinical signs (morbidity 87.27%), and 79 died (mortality 71.82%), after signs of illness (table 1). Among the treated groups, a significant reduction in both morbidity and mortality was observed ($p < 0.05$). Morbidity and mortality among kids of Group II were reduced by 4 and 6 times respectively, in Group III by 3 and 5 times, and in Group IV by 2.5 and 4 times. Accordingly, case fatality in all treated groups was almost half that seen in the control group (table 1).

Except the significantly lower number of kids suffering diarrhoea among treated animals, the duration of scours in each of these groups was significantly ($p < 0.05$) shorter in mean period when compared to those in untreated ones (table 2). The shortest duration of diarrhoea per affected kid was observed in Group II treated with the organic acids (table 2).

Laboratory findings

All nineteen faecal samples examined for coccidia and *Cryptosporidium* spp. were negative at the time of examination. Of the faecal samples cultured on MacConkey and SS agar, no colony showed the phenotypic characteristics of *Salmonella* or *Shigella* spp on SS agar. (Quinn et al., 1994). A total of 43 isolates were identified as MacConkey positive, oxidase and gram-negative rods, but only 36 strains showed from strong to light haemolysis and they were speciated by the API 20E. Three non-haemolytic strains were isolated from cases among the treated groups. The API 20E identified 4 strains as possible *E. coli* (>65%

confidence), and the remaining 32 ones as *E. coli* (94 to 97% confidence). All 36 strains were serotyped with the Fimbrex kits. The kits did not identify any strain as belonging to the 987P antigenic group. They identified 10 strains (27.8%) as belonging to K88 antigenic serotype, 12 strains (33.3%) as belonging to K99 antigenic serotype, 4 strains (11.1%) had evidence from both serotypes and 10 strains (27.8%) were not identified to have any of the examined antigens. Seven of the last 10 strains were isolated from cases observed among the treated groups.

DISCUSSION

Several pathogens that are frequently involved in neonatal kid scours were excluded at present by the clinical signs, history and laboratory examinations. *Salmonella* spp., *Cryptosporidium* sp. and *Eimeria* sp. (not being present in the examined faeces of the kids) usually affect animals older than 5 days of age and may cause haemorrhagic diarrhoea (Navarre et al., 2000; Van Metre et al., 2000). The vaccination history of the does of this farm helped in excluding clostridia as possible causative agents. Besides, infections by clostridia cause symptoms not seen in the situation under study (red-tinged urine and icterus caused by *C. perfringens* type A, or haemorrhagic enteritis and neurological symptoms caused by *C. perfringens* types B and C) (Navarre et al., 2000). No specific examinations were performed in order to exclude rotavirus, an agent known to get quite often involved in kid scours (Legrottaglie et al., 1993; Mendes et al., 1994; Munoz et al., 1995; Gueguen et al., 1996). However, the fact that there was a good protective response to the treatment with antibiotics, indicates that viral diarrhoea was of secondary importance, if at all. ETEC strains, identified in this farm, were therefore, the most likely cause of the scours. Although K99- and F41 positive strains of ETEC are known to cause enteritis in kids, they were never described to induce such severe clinical manifestation as those observed here (Smith and Sherman, 1994; Munoz et al., 1996;

Table 1. Morbidity and mortality of kids during late parturition period after various prophylactic treatments

Treatment group	Health parameters of kids					
	Kids treated (No)	Kids with diarrhoea		Kids died		
		(No)	Morbidity (%)	(No)	Mortality (%)	Case fatality (%)
Group I -control	110	96	87.27 ^a	79	71.82 ^a	82.29 ^a
Group II-acidifier	112	25	22.32 ^b	13	11.61 ^b	52.00 ^b
Group III-ampicillin	105	33	31.43 ^b	16	15.24 ^b	48.48 ^b
Group IV-oxytetrac.	109	40	36.70 ^b	19	17.43 ^b	47.50 ^b

No=Number.

** Definitions: Morbidity= $100 \times (\text{Nr of sick kids} / \text{Nr of total kids in the group})$; Mortality= $100 \times (\text{Nr of kids died} / \text{Nr of total kids in the group})$; Case fatality= $100 \times (\text{Nr of kids died} / \text{Nr of sick kids})$.

^{a,b} Means in the same column with different superscript letters differ ($p < 0.05$).

Table 2. Diarrhoea scores of neonatal kids around during late parturition period after various prophylactic treatments

Treatment group	Diarrhoea scores			
	No of kids with diarrhoea/ No of kids treated	Scouring period of the group (days)	Scouring period per kid±SE (days)	Scouring period per sick kid±SE (days)
Group I-control	96/110	213	1.94 ^a ±0.09	2.22 ^a ±0.05
Group II-acidifier	25/112	36	0.32 ^b ±0.06	1.44 ^b ±0.10
Group III-ampicillin	33/105	57	0.54 ^c ±0.08	1.73 ^c ±0.07
Group IV-oxytetrac.	40/109	71	0.65 ^c ±0.09	1.78 ^c ±0.07

^{a,b} Means in one column with different superscript letters differ ($p < 0.05$).
SE=Standard error.

Van Metre et al., 2000). It could be that differences in management or between strains (e.g. for the first time, here it is reported that K88 ETEC strains can be isolated from caprine diarrhoeas) are responsible for the observed variability.

In this study, organic acids were effective against scours caused by ETEC strains, and their performance was even as good as that of broad-spectrum antibiotics. These findings support similar observations made in pigs suffering from *E. coli* infections (post weaning diarrhoea syndrome, oedema disease), and in such pigs, diarrhoea scores, mortality and growth characteristics (average daily weight gain, feed conversion ratio) were significantly improved after the prophylactic administration of organic acids (Tsiloyiannis et al., 1998a,b). The protective mechanism by which organic acids act is not completely understood. Some believe that acidifiers lower the gastric pH, and thus, they either affect the intestinal micro flora in favor of non-pathogenic fermentative microorganisms, or they increase the enzymic activity of the digestive tract (Ravidran and Korneguy, 1993).

The use of alternative prophylactic methods such as acidifiers in young animals may be of greater importance in certain countries due to their customs. In Greece for example, the meat of young kids and lambs is customarily consumed at the age of 2-3 months. Therefore, drug residues or antibiotic-resistant bacteria can hopefully be reduced in the food of the consumer by such alternative methods.

CONCLUSION

Under field conditions, the preventive administration of organic acids in neonatal kids can protect them from diarrhoea caused by ETEC-strains, sometimes to a similar degree as the administration of broad-spectrum antibiotics.

ACKNOWLEDGEMENTS

The authors wish to thank Mr V. Leontaris, DVM, Mrs. A. Papagiani and Mrs. P. Koukouletsou for their excellent technical assistance.

REFERENCES

- Cooper, V. L. 2000. Diagnosis of neonatal pig diarrhea. In: *Diagnosis of diseases of the digestive tract*. Veterinary Clinics of North America. Food Animal Practice 16:117-133.
- Fallon, R. J. and F. J. Harte. 1980. Effect of feeding acidified milk replacer on calf performance. *Anim. Prod.* 30:459.
- Gueguen, C., A. Maga, M. A. McCrae and G. Bataillon. 1996. Caprine and bovine B rotaviruses in western France: group identification by Northern hybridization. *Vet. Res.* 27:171-176.
- Kyriakis, S. C., V. K. Tsiloyiannis, K. Sarris and J. Vlemmas. 1996a. The effect of Acid Lac Dry in the feed on the metaphylaxis of post-weaning diarrhoea syndrome of piglets. *Proc. 14th International Pig Veterinary Society Congress*, Bologna, Italy, 7-10 July 1996 p. 430.
- Kyriakis, S. C., V. K. Tsiloyiannis, K. Sarris and J. Vlemmas. 1996b. The effect of Acid Lac Dry in the feed on post-weaning oedema disease of piglets. *Proc. 14th International Pig Veterinary Society Congress*, Bologna, Italy, 7-10 July 1996, p. 437.
- Legrottaglie, R., A. Volpe, V. Rizzi and P. Agrimi. 1993. Isolation and identification of rotaviruses as aetiological agents of neonatal diarrhoea in kids. *Electrophoretical characterization by PAGE*. *New Microbiol.* 16:227-235.
- Mendes, V. M., M. C. De Beer, G. H. Goosen and A. D. Steele. 1994. Isolation and preliminary characterization of a caprine rotavirus. *Onderstepoort J. Vet. Res.* 61:291-294.
- Merck Veterinary Manual. 1998a. *Intestinal diseases in pigs*. 8th Ed. (Ed. S. E. Aiello). Published by Merck and Co Inc, Whitehouse Station, N.J., USA. National Publishing Inc, Philadelphia, Pennsylvania. pp. 249-258.
- Merck Veterinary Manual. 1998b. *Intestinal diseases in ruminants*. 8th Ed. (Ed. S. E. Aiello). Published by Merck and Co Inc, Whitehouse Station, N.J., USA. National Publishing Inc, Philadelphia, Pennsylvania, 258-266.
- Munoz, M., M. Alvarez, I. Lanza and P. Carmenes. 1995. An outbreak of diarrhoea associated with atypical rotaviruses in goat kids in Spain. *Res. Vet. Sci.* 59:180-182.
- Munoz, M., M. Alvarez, I. Lanza and P. Carmenes. 1996. Role of enteric pathogens in the aetiology of neonatal diarrhoea in lambs and goat kids in Spain. *Epidemiol. Infect.* 117:203-211.
- Navarre, C. B., E. B. Belkna and S. A. Rowe. 2000. Differentiation of gastrointestinal diseases of calves. In: *Diagnosis of diseases of the digestive tract*. *Vet Clin N Am. Food Anim Pract.* 16:37-57.
- Radostits, O. M., C. G. Gay, D. C. Blood and K. W. Hinchcliff. 1999. Diseases caused by bacteria. In: *Veterinary Medicine: a*

- textbook of the diseases of cattle, sheep, pigs, goats and horses. 9th ed, WB Saunders Co Ltd, London, pp. 753-822.
- Ravindran, V. and E. T. Korneguy. 1993. Acidification of weaner pig diets-A review. *J. Sci. Food Agricult.* 62:313-322.
- Smith, M. C. and D. M. Sherman. 1994. Goat medicine. Lea and Febiger, Philadelphia. pp. 275-358.
- Thomlinson, J. R. and T. L. J. Lawrence. 1981. Dietary manipulation of gastric pH in the prophylaxis of enteric disease in weaned pigs: some field observations. *Vet. Rec.* 109, 120.
- Tsiloyiannis, V. K., S. C. Kyriakis, J. Vlemmas and K. Sarris. 1998a. The effect of organic acids on the control of post-weaning oedema disease of piglets. Proc. 15th International Pig Veterinary Society Congress, Birmingham, England, 5-9 July 1998, p. 151.
- Tsiloyiannis, V. K., S. C. Kyriakis, J. Vlemmas and K. Sarris. 1998b. The effect of organic acids on the control of post-weaning diarrhoea syndrome of piglets. Proc. 15th International Pig Veterinary Society Congress, Birmingham, England, 5-9 July 1998, p. 152.
- Van Metre, D. C., J. W. Tyler and S. M. Stehman. 2000. Diagnosis of enteric disease of small ruminants. In: *Diagnosis of diseases of the digestive tract.* *Vet Clin N Am, Food Anim Pract* 16:87-115.

