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FALLIBILITY OF FAECAL CONSISTENCY AS A CRITERION OF SUCCESS IN THE EVALUATION OF ORAL FLUID THERAPY FOR CALF DIARRHOEA

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SUMMARY

It is often said that the success of oral rehydration in humans depends on the adequacy of the improvement in the composition and volume of extracellular fluid, not reduction of faecal output. Indeed, the latter may increase initially. Such increases do not prevent the treatment from being effective but they may, falsely, undermine its acceptability to patients or those caring for them. This paper provides data to show that standard oral rehydration solutions used to treat experimentally induced calf diarrhoea procure identical improvements in plasma volume during the first 48 h, whether faeces improve or not, and those calves whose faecal consistency improved actually showed greater deterioration of extracellular fluid volume. While it is important for this to be appreciated by clinicians and explained to owners, it is absolutely imperative that those responsible for the approval of new therapeutic products for registration understand and accept that faecal consistency offers no reliable insight into the effectiveness of oral rehydration therapy for calf diarrhoea. It was, however, interesting that there was some relationship with correction of acidosis—perhaps because some of the contributing factors arise from colonic dysfunction.

KEYWORDS: Oral rehydration; efficacy; diarrhoea; faecal output; product registration/licensing.

INTRODUCTION

The primary object of oral rehydration therapy (ORT) in the treatment of diarrhoea is to correct the fluid and electrolyte depletion and the acid–base disturbances, allowing the patient's natural defences to overcome the cause of the diarrhoea and to restore optimal conditions for the normal gut flora (Michell, 1983, 1994). Specifically, the aim is to treat the patient rather than the faeces (Ludan,

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1988; Avery & Snyder, 1990). The most important single objective is to restore plasma volume and reduce the danger of circulatory shock.

In human medicine, from the earliest days of ORT, there have been problems with resistance from patients (or their mothers) if the faeces failed to improve, or if faecal loss even increased, in the early stages of therapy (Carpenter, 1987; Naylor, 1990; Ribeiro & Lifshitz, 1991). Indeed, rehydration may increase the patient's ability to produce fluid faeces as well as restoring circulating volume. It required persistent education by health workers to persuade mothers that this did not matter provided the child's condition was improving. Naturally, it implies that utilization of the oral rehydration solution (ORS) is less than perfect, but the perfect ORS is yet to emerge in either human or veterinary medicine.

In veterinary medicine, the analogous problem is obviously important: faced with a tardy improvement in faecal appearance, the farmer or the veterinarian may well conclude that they should try something different. Worse, the European Commission (EC) sets particular store by clinical criteria, notably improvement of faecal consistency (as reflected in faecal score (FS)), in judging the suitably of new oral rehydration products for registration (EC, 1992). Yet we have shown that clinical criteria, including faecal consistency, are hopelessly insensitive as a basis for comparing the efficacy of different ORSs (Michell *et al.*, 1992). We therefore thought it important to examine the particular question 'Does improvement of faecal appearance, offer even a crude guide to the effectiveness of an ORS in restoring plasma volume?'. We thought it important to use mainstream, conventional ORT rather than novel solutions to answer it, hence our choice of the two most widely used veterinary oral rehydration products in the UK for our treatment. The focus of the study, however, is not the effectiveness of the products but the prognostic value of faecal improvement in gauging the success of oral rehydration therapy.

METHODS

General

Our methods have been published in detail (Michell *et al.*, 1992), including the composition of the two solutions used (Lectade or Lectade Plus, SmithKline Beecham). In brief, calves were purchased from their original farms within 24 h of birth and any showing spontaneous diarrhoea following arrival were excluded. Diarrhoea was induced by using enterotoxigenic *Escherichia coli* (ETEC) (Bywater, 1977; Groutides & Michell, 1990; Michell *et al.*, 1992) and treated by standard oral rehydration therapy. Measurements were made before induction of diarrhoea and before and during its treatment. The results of these measurements were compared in calves showing or failing to show improvement in faecal consistency during treatment.

Management of calves

The calves (dairy or dairy-cross) were weighed on arrival (day A) and individually penned on wood chips. They were offered warm milk substitute (Volac Easimix) twice daily (08.00 and 16.00 h), starting on the evening of day A and

were encouraged to drink from the bucket as soon as possible, either with or without a teat.

Calves were observed twice daily at feeding times from the morning of day B and faecal consistency was scored (see below). There was a qualifying period covering three observations. Many calves had transient high scores (three or four) as meconium was eliminated and as diet changed to skimmed milk powder, but most had normal faeces by the morning of day C. Calves with a cumulative FS above five by the morning of day C were withdrawn from the trial.

FSs were assessed twice daily as follows: 1=firm; 2=semi-solid; 3=liquid; 4=very liquid.

On day C, blood samples were taken from calves to obtain baseline values of the plasma parameters to be monitored during treatment and to assess the transfer of colostral immunoglobulin to each calf using the zinc sulphate turbidity (ZST) test (McEwan *et al.*, 1970).

Induction of diarrhoea

On days C and D, prior to feeding, each qualifying calf was drenched with 4 g of sodium bicarbonate in 60 ml water to overcome the protective effects of abomasal acidity. Calves were then dosed with 10^{10} ETEC (09:K30:K99) in 10 ml sterile brain/heart infusion broth culture (a modification described by Bywater, 1977). Aliquots from a stock culture of ETEC were stored at −20°C. These samples were then used to seed 10 ml broth cultures, which were incubated at 39°C for 16 and 24 h, respectively.

Treatment of calves

The day that ORS treatment commenced became day V; subsequent days of treatment were days W to Z inclusive. Treatment of calves began when the cumulative faecal score reached 10 within 24 h of the first inoculation or 13 within 3 days (or intermediate values; Michell *et al.*, 1992). Treatment began at the next afternoon feed-time (now day V) following the qualifying score and either Lectade or Lectade Plus was used in accordance with the manufacturer's instructions (2 l twice daily for 48 h; followed by 2 l of equal volumes of ORS and milk replacer twice daily for 48 h). Faecal scoring continued throughout the experiment and no other treatments were given.

Determination of fluid volumes and electrolyte and acid–base changes

Plasma and extracellular fluid (ECF) volume were measured on the day of inoculation (day C), on the day that diarrhoea reached the qualifying criterion for therapy (day V) and at 24 and 48 h into the treatment period (days W and X). We examined the response to treatment (improvement of plasma or ECF volume in the first 48 h of ORT) in two groups of calves: '*improvers*', selected because they had the greatest reduction of FS (2 or more; n=15) or '*non-improvers*' with faecal scores that failed to improve or deteriorated during the same period (n=7). We also looked at changes in acid–base balance, using our published methods (Michell *et al.*, 1992). Differences were evaluated using Student's t test, with $P<0.05$ considered significant.

Equal volumes of 10% (w/v) sodium thiocyanate (NaSCN) and 1% (w/v) Evans

blue dye (EB) were mixed and sterilized in 100 ml volumes. At 14.00 h on day C, before the afternoon feed, an i.v. cannula (Critikon IV Cannula, Critikon Ltd, 18 G) was inserted into the jugular vein of the calf and a 10 ml heparinized blood sample (Monovette Li-Heparin LH/10, Sarstedt) was collected. Immediately after this a standard 5 ml volume of the NaSCN and EB mixture was given *via* the cannula. The cannula was flushed two or three times by aspiration and reinjection of blood. A digital stopwatch was started and the cannula was removed.

Sampling. Serial blood samples were taken *via* single-use hypodermic needles (Monoject, 19G × 1 inch) and heparinized syringes (as before) after 15, 30, 60 and 120 min. The pre-injection sample provided a blank. This procedure was repeated on days V, W, X and Y. Samples were centrifuged (with 1 ml Serasieve; Hughes & Hughes Ltd) at 3500 r.p.m. for 15 min, within 60 min of collection.

Analyses. (1) Plasma volume determination using Evans blue dye method. The optical density (OD) of plasma samples I to IV was read immediately (SP 30 Spectrophotometer, Pye Unicam) against a water blank, at 620 nm. The OD values were compared with a standard aliquot of the injection solution diluted 1:1000 in 5% (w/v) albumin, giving a final Evans blue concentration of 5 mg l^{-1}.

(2) ECF volume using NaSCN method. A volume of 0.5 ml plasma was mixed with 0.5 ml of 20% (v/v) trichloroacetic acid, the mixture was shaken well and centrifuged (3000 r.p.m., 10 min). A sample of 100 µl of the supernatant was mixed with 400 µl of 16% (w/v) ferric nitrate made up in *N*-nitric acid; OD was read immediately at 460 nm. The procedure was carried out on a Gilford selective batch analyser (SBA 300). The standard was analogous to that for Evans blue. Results were calculated as described in Michell *et al.* (1992).

Fluid spaces obtained by these techniques are extremely similar in calves to those obtained by the use of radiolabelled sodium or albumin, respectively (Wagstaff *et al.*, 1992).

(3) Determination of plasma sodium, potassium concentration, acid–base status. Heparinized blood samples taken anaerobically on days C, V, W, X and Y were placed on ice until they reached the laboratory (within 1 h) for analysis (pH, pCO_2, bicarbonate (BIC), Corning 158 Blood Gas Analyser). Plasma was separated and stored deep frozen (−20°C) for measurement of plasma sodium (Na) and potassium (K) using an integrating flame photometer (Radiometer FLM3). Our usual quality controls (Groutides & Michell, 1990) were used. In addition, a single blood sample was taken on day Z for Na and K determination.

RESULTS

The number of calves treated with Lectade or Lectade Plus was comparable in both groups (improvers, n=7 and 8: non-improvers, n=3 and 4, respectively). Prior to diarrhoea, plasma volume was identical in improvers and non-improvers (Table I) and the fall prior to therapy was indistinguishable (0.46 l and 0.44 l). There were no significant differences between groups in any other variable prior to induction of diarrhoea (Table I). The plasma volume at the start of treatment (Table II) was the same in both groups (improvers, 2.3(±0.1) l; non-improvers

2.4(±0.2) l (mean±SEM). There was no statistically significant difference between groups in any of the variables at the start of treatment (Table II). The increase in plasma volume during the first 48 h of therapy (Table III) was identical, whether or not faecal consistency improved (0.1(±0.1) l and 0.1(±0.1) l). ECF volume did not increase during the first 48 h of ORT and there was a fall, which was statistically indistinguishable between improvers (0.6(±0.4) l) and non-improvers (0.3(±0.6) l), although it was larger in the former group. Improvers did, however, have a significantly more favourable change in plasma bicarbonate (+3.9(±1.9) mmol l^{-1}) compared with non-improvers (−2.7(±0.4) mmol l^{-1}; $p<0.05$).

Table I
Characteristics of 'improvers' and 'non-improvers' before induction of diarrhoea

Variable	*Improvers*	*Non-improvers*
Plasma volume (l±SEM)	2.8±0.6	2.8±0.5
ECF volume (l±SEM)	11.3±0.8	10.5±1.0
Blood pH (units±SEM)	7.377±0.016	7.412±0.014
Blood [BIC] (mmol l^{-1}±SEM)	38.0±0.9	38.5±1.9
Plasma [Na] (mmol l^{-1}±SEM)	140.4±0.6	139.9±0.9
Plasma [K] (mmol l^{-1}±SEM)	5.5±0.3	5.5±0.4

Table II
Characteristics of 'improvers' and 'non-improvers' before treatment of diarrhoea by ORT

Variable	*Improvers*	*Non-improvers*
Plasma volume (l±SEM)	2.3±0.13	2.4±0.20
ECF volume (l±SEM)	9.7±0.88	8.5±0.70
Blood pH (units±SEM)	7.347±0.022	7.365±0.023
Blood [BIC] (mmol l^{-1}±SEM)	30.0±2.0	34.2±1.5
Plasma [Na] (mmol l^{-1}±SEM)	135.7±1.4	136.4±1.7
Plasma [K] (mmol l^{-1}±SEM)	5.6±0.2	5.7±0.1

Table III
Fluid and electrolyte changes in response to 48 h ORT (100%) in 'improvers' and 'non-improvers'

Variable	*Improvers*	*Non-improvers*
Plasma volume (l±SEM)	+0.11±0.06	+0.11±0.11
ECF volume (l±SEM)	−0.64±0.43	−0.34±0.64
Blood pH (units±SEM)	+0.033±0.023	+0.018±0.029
Blood [BIC] (mmol l^{-1}±SEM)	+3.9±1.87	−2.7±0.38
Plasma [Na] (mmol l^{-1}±SEM)	+3.2±1.0	+0.3±1.6
Plasma [K] (mmol l^{-1}±SEM)	+0.4±0.2	+0.6±0.2

DISCUSSION

The calves with the greatest improvement of faecal consistency had no greater increase in plasma volume than calves which did not improve their faecal consistency in response to ORT; moreover, the calves with improved faecal consistency showed a greater fall in ECF volume during treatment (although this difference was not statistically significant). The data confirm the clinical observations from human oral rehydration that improvement of the faeces offers no guide to the rehydration of the patient. There are numerous reasons why this should be so. First, it does not matter if an ORS slightly increases faecal loss if it considerably improves the volume and composition of plasma and extracellular fluid. Second, FSs, though widely used, are qualitative rather than quantitative and need not accurately reflect the daily loss of either water or electrolytes. Third, even faecal output does not necessarily reflect current events within the intestine; calves may die of fluid loss into the gut before it has time to appear as diarrhoeic faeces, especially if there is decreased intestinal motility and enteric pooling. Similarly, increased faecal output during therapy may reflect unfavourable exchanges between gut and plasma that actually occurred before treatment began.

The difference in acid–base balance is interesting; its emergence despite the absence of differences in rehydration may well reflect the likelihood that, in part, the acidosis is caused by events within the colon (Grove-White & White, 1993). These could, therefore, relate to visible changes in the faeces. The fact remains, however, that effectiveness of correction of acidosis can only be reliably assessed by measurement of changes in plasma pH and/or bicarbonate, not by the examination of faeces.

Diarrhoea is the outcome of complex interactions between the patient, its diet, the form and function of its intestinal villi and the gut flora. Thus, even with a known 'trigger', the actual causes and their interactions can vary greatly between individuals. It is, therefore, difficult to devise consistent models and extremely difficult to contend with the multiple sources of adventitious variation in field studies. These problems are hard enough to address in attempting to assess real improvements in oral rehydration therapy, without placing unfounded reliance on ineffective or misleading criteria such as changes in faecal consistency. While it is important for this to be appreciated by those who care for clinical cases, it is even more vital for it to be understood by those who exert legislative control over the future supply of new therapeutic products.

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