## PATHOPHYSIOLOGY OF SEVERE DIARRHOEA AND SUGGESTED INTRAVENOUS FLUID THERAPY IN CALVES OF DIFFERENT AGES UNDER FIELD CONDITIONS

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#### Abstract

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Selected clinical and biochemical variables were studied in 2 groups of severely dehydrated, diarrhoeic calves before and after fluid therapy. Group 1 (n = 10) included calves aged 2 to 7 d, Group 2 (n = 9) calves aged 8 to 14 d. Clinical examination and blood sampling for biochemical and haematological analyses were performed in each animal before and after fluid therapy. Samples of faeces for microbiological examination were taken before intravenous (IV) rehydration. Solutions used for IV rehydration contained salts of NaCl, NaHCO<sub>3</sub>, KCl and glucose in different quantities for calves in Groups 1 and 2. These rehydration solutions were infused IV at a volume of 4 l to each calf during 3 hours and then followed by oral rehydration. In Group 1, rotavirus in faeces was diagnosed in 50.0 % of all calves; combined infectious agents rotavirus and *Cryptosporidium* spp. occurred in 10.0 %, none of the calves had in faeces coronavirus, enterotoxigenic *E. coli* K99 and *Salmonella* spp.; that means in 40% of calves of this group no infectious agents rotavirus and *Cryptosporidium* spp. were diagnosed in 55.5%. none of the calves had enterotoxigenic *E. coli* K99 or *Salmonella* spp in faeces.

Before IV rehydration diarrhoeic calves in Group 2 presented mean blood values of pH 7.12, base excess (BE) -14.72 mmol/l, standard bicarbonate (SB) 9.86 mmol/l which were significantly lower (P<0.05) than in calves in Group 1 before IV rehydration, where pH was 7.16, BE -11.68 mmol/l and SB 12.54 mmol/l. In both groups of calves before IV rehydration these low values of blood pH, BE, SB and normal value of  $pCO_2$ , corresponded to partially compensated metabolic acidosis. The differences in blood values of pH, BE, SB, PCV, urea and K in both groups before and after rehydration were significant (P<0.01). The metabolic acidosis together with hyperkalemia, prerenal uremia and haemoconcentration were restored after fluid therapy. Rehydration was successful in 79.0% of all diarrhoeic calves. Calves aged 8 d or older suffering from severe diarrhoea needed more bicarbonate for IV rehydration and volume of solutions for IV rehydration used in this study simplify fluid therapy in severely diarrhoeic calves of different ages in lateral or sternal recumbency without suckling reflex and under field conditions.

Neonatal diarrhoea, dehydration, metabolic acidosis, hyperkalemia, uremia, packed cell volume, fluid therapy, calf

Diarrhoea with dehydration is frequent in calves during the first 3 weeks of their life, and it is the most important cause of morbidity and mortality. The most commonly recognized causes of neonatal calf diarrhoea are rotavirus, coronavirus, Cryptosporidium spp., enterotoxigenic E. coli and nutritional abnormalities (Salajka and Ulmann 1972; Acres et al. 1977; Reynolds et al. 1986; Snodgrass et al. 1986; Bouda et al. 1990). When solving the problem of neonatal death losses in dairy calves it is necessary to evaluate their colostral immunity (immunoglobulin status), and feeding, housing, management, disinfection, health status and vaccination programs in their dams (Medina 1994; McGuirk 1996; Meltzer and Shpigel 1996).

In pathogenesis of diarrhoea the most important changes occur in the function of the intestinal mucosa. Diarrhoea is a result of either increased secretion or decreased absorption in the intestine. Bacteria such as enterotoxigenic *E. coli*, and to some extent *Salmonella* spp. cause neonatal diarrhoea by secreting enterotoxins, which stimulate increased intestinal secretions (Argenzio 1985; Bywater 1977; Fromm et al.1974). These changes are mediated by the cyclic adenosine monophosphate or cyclic guanosine 4,5-monophosphate, calmodulin and changes in protein kinase activity (Argenzio 1985). Cell structure is not affected, but the activity of membrane pumps is altered, and therefore, the secretion of sodium, chloride and potassium is increased (Bywater and Logan 1974).

Enteric viruses (rotavirus, coronavirus) and protozoa (*Cryptosporidium* spp.) cause neonatal diarrhoea as a result of destruction of the absorptive villous epithelial cells. Diarrhoea due to rotavirus and coronavirus follows because intestinal secretion continues, while absorption is impaired (Mebus et al. 1973; Moon 1978; Woode et al. 1978). The osmotic effect of unabsorbed nutrients drains water into the gut and further aggravates diarrhoea (Acres 1985). Inflammation of the intestinal mucosa is a sign of salmonellosis and clostridiosis, which contributes to diarrhoea by increasing mucosa pore size and hydraulic pressure in the intestinal wall by destroying the absorptive cells and by increasing prostaglandin production, which stimulates mucosal secretory mechanisms (Argenzio 1985).

Metabolic changes associated with neonatal diarrhoea in calves have been studied extensively. Major disturbances in diarrhoeic calves are dehydration, metabolic acidosis, electrolyte abnormalities and uremia. Fluid is lost preferentially from the vascular space (Naylor 1987; Bouda et al.1994).

The objective of this work was to study the pathophysiology of severe diarrhoea in calves from 2 to 7 d old and calves between 8 to 14 d old, and their response to intravenous (IV) fluid therapy.

#### Materials and Methods

We used 19 Holstein-Friesian calves aged 2 to 14 d suffering from profuse diarrhoea and severe dehydration. They were located in 2 farms: one farm had a capacity of 420 and the second of 600 dairy cows. All dry cows were vaccinated against *E. coli K99*. After birth calves were separated from their dams and placed indoors in individual calf boxes. They were offered by bottle 2 l of colostrum from their mothers twice a day during the first 4 d of life, and thereafter they were fed milk or commercial milk replacer. The calves were divided into 2 groups by age. In Group 1 (n = 10) calves were 2- to 7-d-old, in Group 2 (n = 9) calves 8- to 14-d-old. The calves were weighed at birth, three times a week, and before and after IV rehydration. The mean body mass of calves at birth in Group 1 was 38.2 kg (36.2 to 41.1) and in Group 2 it was 39.3 kg (37.4 to 43.7). At clinical examination we registered: respiratory and cardiac frequencies, body temperature, behaviour, presence or absence of suckling reflex, severity of diarrhoea, severity of dehydration (sunken eyes, elasticity of the skin, colour and humidity of mucosas) and physical status (standing, sternal or lateral recumbency). There was profuse diarrhoea, severe dehydration and absence of suckling reflex in both groups in either sternal or lateral recumbency in all animals studied.

Blood samples for biochemical and haematological analyses were collected from the jugular vein before IV rehydration and 48 hours thereafter. Indicators of acid-base status (pH, partial pressure of carbon

dioxide-pCO<sub>2</sub>, base excess-BE, standard bicarbonate-SB) were determined by means of the Acid-base analyzer-ABL 3, Radiometer (Copenhagen) in heparinized blood samples, within 3 h of collection. In several animals, analyses were done 4 to 12 h after the collection of the samples. Therefore, correcting factors were used for acid-base results (J a g o š et al. 1977). Packed cell volume (PCV) was determined by microhaematocrit methods. The following indicators were determined in blood plasma: urea and glucose by the Analyzer Micro-chem 565 (Ciba Corning) Na and K were determined by atomic absorption spectrophotometry (Perkin-Elmer 3110). All samples of faeces for microbiology examination, (enterotoxigenic *E. coli* K99, *Salmonella* spp., rotavirus, coronavirus and *Cryptosporidium* spp.) were taken before rehydration.

For IV rehydration we used isotonic sterile solutions with salts of NaCl, NaHCO<sub>3</sub>, KCl and slightly hypertonic solution of glucose. The composition of the solutions for IV rehydration was based on preliminary experimental results using 10 diarrhoeic calves. The composition of the infused solutions for Group 1 was as follows: 18 g of NaCl (21 of 0.9 % NaCl), 17 g of NaHCO<sub>3</sub> (1.31 of 1.3 % NaHCO<sub>3</sub>), and slightly hypertonic solution of 50 g of glucose (0.51 of 10% glucose), 2.2 g of KCl (200 ml of 1.1 % KCl) making a total volume of 4.0 l. The composition and volume of the infused solution for Group 2 was: 13.5 g of NaCl (1.51 of 0.9 % NaCl), 2.3 4 g of NaHCO<sub>3</sub> (1.81 of 1.3 % NaHCO<sub>3</sub>), 50 g of glucose (0.51 of 10% glucose), 2.2 g of KCl (200 ml of 1.1 % KCl), making a total of 4.0 l.

The first liter of warmed IV rehydration solution was infused within 30 minutes using IV plastic catheters, and the remaining 3.01 were infused within approximately 2.5 hours.

The IV rehydration was followed by oral rehydration which consisted of 4.2 g of NaCl, 4.0 g of NaHCO<sub>3</sub>, 1.6 g of KCl and 20 g of glucose dissolved in 1 l water. The total volume of oral fluids per calf/day was 6 l divided in 3 equal doses.

Milk or milk replacer was witheld from the diet of calves for a period of 36 to 48 h. The treatment of dehydrated diarrhoeic calves in addition to IV and oral rehydration, included oral antibiotic therapy for 3 days (2 calves in Group 1 and 1 calf in Group 2) and symptomatic therapy consisting of warming of the body using bags with luke-warm water or infrared lamps.

Results were statistically analysed by the Student's t- test and analysis of variance, and were expressed as means + SD.

#### Results

Calves from both groups were severely dehydrated and showed no suckling reflex. In Group 1, 5 calves were in sternal and 5 in lateral recumbency. In Group 2, 6 calves were in sternal and 3 in lateral recumbency. Clinical signs of dehydration, especially enophtalmus were more severe in calves of Group 1. Body mass losses made the difference between the last weighing and the weighing before the moment of rehydration. The loss of body mass for Group 1 of calves was 9.76% (8.1-10.5%), and for Group 2, 9.32% (8-10.2%). Body temperature in Group 1 was normal in 4 calves and decreased in 6 calves; in Group 2 it was normal in 5 and decreased in 4 calves.

In Group 1, rotavirus in faeces was diagnosed in 50.0 % of all calves; combined infectious agents rotavirus and *Cryptosporidium* spp. occurred in 10.0 %, none of the calves had coronavirus, enterotoxigenic *E. coli* K99 and *Salmonella* spp. in faeces. In 40.0% of calves no infectious agents were isolated in faeces. In Group 2, coronavirus was found in 11.1 %, combined infectious agents rotavirus and *Cryptosporidium* spp. were diagnosed in 55.5 % and none of the calves had enterotoxigenic *E. coli* K99 or *Salmonella* spp. in faeces. In 33.4 % of calves, no infectious agents were isolated from faeces.

The most important pathophysiological changes in all calves of both groups before IV rehydration were profuse diarrhoea, severe dehydration, very low blood pH, BE, SB and increased PCV, plasma urea and K concentrations. The biochemical indices in heparinized blood and plasma of calves before and after IV rehydration are presented in Tables 1 and 2.

The decrease in pH, BE and SB before IV rehydration was more evident in calves of Group 2 than in calves of Group 1, and this difference was significant (P < 0.05).

Before IV rehydration							
Group of calves	рН	BE* mmol/l	SB* mmol/l	pCO <sub>2</sub> * KPa	PCV* (I/I)		
1 (n = 10)	7.161	-11.68	12.54	5.21	0.44		
	0.053	2.75	2.67	0.55	0.07		
2 (n = 9)	7.123	-14.72	9.86	5.43	0.42		
	0.061	3.43	3.22	0.67	0.06		
	P < 0.05	P < 0.05	P < 0.05	N. S.	N. S.		
		After IV rehydi	ration (48 hours)				
1 (n = 8)	7.357	2.17	25.89	5.77	0.32		
	0.04	2.98	2.87	0.65	0.05		
2 (n = 7)	7.348	1.35	25.13	5.55	0.33		
	0.053	2.93	2.74	0.69	0.06		
	N. S.	N. S.	N. S.	N. S.	N. S.		

 Table 1

 Values of acid-base status and PCV in calves before IV rehydration

\* BE = base excess, SB = standard bicarbonate, pCO<sub>2</sub> = partial tension of CO<sub>2</sub>, PCV = packed cell volume.

Before IV rehydration							
Group of calves	Urea	Glucose	Na	K			
	mmol/l	mmol/l	mmol/l	mmol/l			
1 (n = 10)	13.8	5.12	134.4	5.72			
	5.1	1.60	7.2	1.01			
2 (n = 9)	14.2	4.48	137.3	5.81			
	5.32	1.45	8.6	1.25			
	N. S.	N. S.	N. S.	N. S.			
		After IV rehydration (48 h	nours)				
1 (n = 8)	5.3	5.03	139.6	4.94			
	1.7	1.2	6.3	0.77			
2 (n = 7)	5.7	4.87	142.5	5.07			
	1.9	1.3	7.3	0.58			
	N. S.	N. S.	N. S.	N. S.			

 Table 2

 Biochemical values in blood plasma of calves before and after IV rehydration

Values of  $pCO_2$ , PCV, urea, glucose, Na and K in both groups of diarrhoeic calves before rehydration were not different. Severe metabolic acidosis (partially compensated), hyperkalemia, prerenal uremia and hemoconcentration were observed in diarrhoeic calves before IV rehydration. The differences in blood values of pH, BE, SB, PCV, urea and K in both groups of calves before and after rehydration were significant (P<0.01). After IV and oral rehydration, biochemical values in blood were restored and there were no significant differences in these variables between Groups 1 and 2. The IV rehydration combined with oral rehydration was successful in 15 out of 19 calves (79.0%). The therapy efficiency was estimated on the base of clinical examination of the animals and comparison of biochemical values in blood before and after rehydration. The causes of death in 2 calves of Group 1 and in 2 calves of Group 2 were severe metabolic acidosis, dehydration and uremia.

### Discussion

Diarrhoea associated with dehydration, metabolic acidosis and electrolyte abnormalities are the main health problems in calves up to 3 weeks of age. From this study, it is evident that the important etiologic agents causing diarrhoea in calves aged 2 to 14 d are: rotavirus, *Cryptosporidium* spp. and coronavirus. Similar findings were described by Reynolds et al. (1986) and Snodgrass et al. (1986). Negative microbiological findings were obtained in 36.8 % of all diarrhoeic calves. Other possible causes of diarrhoea are non-infectious such as feeding high volumes of milk at once, low quality milk replacers, feeding cold milk, calving or rearing in droughty or humid housing conditions.

Dehydration in diarrhoeic calves results from faecal fluid loss and decreased fluid intake due to anorexia or withdrawal of milk. Faecal volume in diarrhoeic calves can reach as much as 13 % of their body mass in 24 h as compared to 0.3 % in non-diarrhoeic calves (Lewis and Phillips 1972). In our study the degree of dehydration and changed PCV were more evident in diarrhoeic calves under 7 d of age than in older calves; these findings correspond to those of Naylor (1987, 1996), Roussel and Kasari (1990). According to Roussel and Kasari (1990) the experienced veterinarian can classify dehydrated calves by assessing clinical signs such as enophtalmus, decreased skin elasticity, lack of suckling reflex, posture and surface temperature of the extremities. These dehydrated calves can be classified in two groups: 1) animals requiring intravenous rehydration therapy and 2) animals that will respond to oral rehydration. Intravenous rehydration is indicated in calves suffering from over 8% dehvdration (Michel et al. 1989; Radostits et al. 1994; Medina 1994). Values of PCV were significantly increased in diarrhoeic calves before IV rehydration, as compared with those after rehydration. This agrees with the reference values stated by Bouda and Jagoš (1984).

Metabolic acidosis is a consistent complication of diarrhoea caused by bicarbonate loss in the diarrhoeic faeces, by lactic acid accumulation in poorly perfused tissues, by reduced acid excretion in poorly perfused kidneys and by organic acid production in the colon as a result of fermentation of unabsorbed nutrients (Roussel and Kasari 1990; Bouda and Jagoš 1991; Medina 1994).

A fast technique to estimate the degree of metabolic acidosis is to use acid-base analyzers if they are available. The determination of base excess in blood is especially important for the treatment of metabolic acidosis. Bicarbonate requirements for neutralizing acidosis in calves can be calculated from the Mellemgaard-Astrup equation as follows:

Bicarbonate requirements (mmol) = BE (mmol/l)  $\times 0.5 \times$  body mass (kg).

One g of NaHCO<sub>3</sub> equals to 12 mmol of bicarbonate or 1.0 ml of a 8.4 % solution of NaHCO<sub>3</sub> equals to one mmol of bicarbonate (Kasari 1990; Bouda et al. 1994, Radostits et al. 1994;). The estimation of the acid-base status in diarrhoeic calves

over 7 d old on the basis of clinical signs of dehydration, is less reliable than in calves younger than 7 d (Naylor 1996). From our results, it is evident that the severely dehydrated calves over 1 week of age presented more severe metabolic acidosis (BE = -14.72 mmol/l) than the younger ones (under 7 days old, BE = -11.68 mmol/l). Very similar results are described by Naylor (1987, 1996). Diarrhoeic calves younger than 7 d in sternal or lateral recumbency, suffering from severe dehydration and showing no suckling reflex, with determined BE = -11.68 mmol/l, will need 209 mmol of NaHCO<sub>3</sub> (17.4 g of NaHCO<sub>3</sub>) or 1.3 l of a 1.3 % solution of NaHCO<sub>3</sub>. On the other hand, calves over 7 d of age with BE = -14.72 mmol/l need to neutralize the metabolic acidosis, 279 mmol of NaHCO<sub>3</sub> (23.5 g of NaHCO<sub>3</sub> or 1.8 l of a 1.3 % solution of NaHCO<sub>3</sub>.

Concentrations of plasma electrolytes provide the assessment of their content in the extracellular compartment but they do not offer good information on the total electrolyte content in the body of the animal (Bouda et al. 1990; Roussel and Kasari 1990). Before IV rehydration there was hyperkalemia in both groups of diarrhoeic calves as compared with the values after IV rehydration or the reference values stated by Bouda and Jagoš (1984). Hyperkalemia occurs in severely diarrhoeic calves, because H ions diffuse from acid plasma into the intracellular fluid compartment and in order to maintain electroneutrality, K ions diffuse into the plasma. Actually, the total body K content is decreased, because it is lost through the diarrhoeic faeces and the renal excretion. Therefore, salts of KCl were added to the IV rehydration solution. The quantity of added KCl in our IV solutions was lower than that in solutions recommended by Roussel and Kasari (1990). In spite of the addition of salts of KCl to the IV rehydration solutions for hyperkalemic diarrhoeic calves, plasma levels after IV rehydration were restored. In our opinion it is convenient to add KCl to IV rehydration solutions in the dose as we have done. Plasma concentrations of sodium in calves before and after IV rehydration were in the range of reference values determined by Bouda and Jagoš (1984) but the total body content of sodium was reduced in diarrhoeic calves due to severe dehydration. In dehydrated diarrhoeic calves, it is important to replace the extracellular volume with solutions containing Na ions at the same concentrations as in the blood plasma. Plasma glucose concentrations below the reference values (Bouda and Jagoš 1984) were only found in diarrhoeic calves over 7 d of age. According to Roussel and Kasari (1990), the addition of glucose to rehydration solutions includes these benefits: glucose is the source of energy especially for hypothermic, hypoglycemic calves, and it also stimulates the release of insulin which in turn enhances the movement of K from the extracellular compartment to the intracellular compartment.

The success of the treatment of diarrhoea depends on the etiologic agents, clinical signs of diarrhoea, degree of dehydration, age of the calves, composition and doses of rehydrating solutions including the antibiotic and symptomatic therapy (B ou d a et al. 1994; R a dostits et al. 1994). The infectious agents, antibiotic and symptomatic therapy in both groups of calves were in our study practically similar, therefore we assume that the main cause of the severe metabolic acidosis in Group 2 was the age of animals and these findings are in agreement with the results obtained by N a ylor (1987, 1996). The suggested composition and volume of solutions for IV fluid therapy used in this study simplify the fluid therapy in calves of different ages (up to 7 d and over 7 d old) with severe diarrhoea, showing no suckling reflex and in recumbency under field conditions.

# Patofyziologie těžkých průjmů a navržená intravenózní rehydratace u telat různého věku v podmínkách praxe

Vybrané klinicko-biochemické ukazatele byly studovány u 2 skupin telat s těžkými průjmy před a po rehydratační léčbě. V 1. skupině (n = 10) byla telata ve věku 2 až 7 dní, ve 2. skupině (n = 9) 8 až 14 dní. Klinické vyšetření a odběr krevních vzorků pro biochemické a hematologické vyšetření bylo provedeno u všech zvířat před intravenózní (i.v.) a po rehydratační léčbě. Mikrobiologické vyšetření vzorků trusu bylo provedeno před i.v. rehydratací. Použité roztoky pro i.v. rehydrataci obsahovaly soli NaCl, NaHCO<sub>3</sub>, KCl a glukózu v rozdílných dávkách pro telata 1. a 2. skupiny. Tyto roztoky v objemu 4 l byly aplikovány i.v. během 3 h s následnou perorální rehydratací. V trusu 1. skupiny byl zjištěn rotavirus u 50,0 % zvířat; rotavirus a *Cryptosporidium* spp. byly současně nalezeny u 10,0 %, koronavirus, enterotoxigenní *E. coli* K99 a *Salmonella* spp. nebyly nalezeny u žádného telete, což znamená, že u 40,0 % telat této skupiny nebyly v trusu zjištěny infekční agens. V 2. skupině telat byl koronavirus v trusu zjištěn u 11,1 %, rotavirus a *Cryptosporidium* spp. byly současně diagnostikovány u 55,5 %; u žádného telete této skupiny nebyly v trusu zjištěny enterotoxigenní *E. coli* K99 a *Salmonella* spp.

U průjmujících teľat 2. skupiny byly před i.v. rehydratací průměrné hodnoty pH krve 7,123, base excess (BE) -14,72 mmol/l, standardního bikarbonátu (SB) 9,86 mmol/l významně nižší (P < 0,05) než u průjmujících telat 1. skupiny pred i.v. rehydratací, kde pH krve bylo 7,161, BE -11,68 mmol/l, SB 12,54 mmol/l. Velmi nízké hodnoty pH krve, BE, SB a normální hodnota pCO<sub>2</sub> krve svědčí pro částečně kompenzovanou metabolickou acidózu. V hodnotách pH, BE, SB, hematokritu, močoviny a K byly u obou skupin telat před rehydratací a po rehydrataci signifikantní rozdíly (P < 0.01). Metabolická acidóza doprovázena hyperkalémií, prerenální urémií a hemokoncentrací byly normalizovány použitou rehydratační léčbou, která byla úspěšná v 79.0 % všech telat s těžkým průjmem. Taková telata ve věku nad 7 dní potřebují při i.v. rehydratačních roztoků pro i.v. aplikaci, použité v této studii, výrazně přispívají ke zjednodušení i.v. rehydratační terapie u telat s těžkým průjmem bez sacího reflexu, bez schopnosti povstat, zejména v podmínkách praxe.

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