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Physiopathology of diarrhea in young calves: clinical signs and metabolic disturbances

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Abstract - The aims of the current study were to characterize the main clinical consequences of diarrhea and to identify metabolic disorders due to this neonatal disease in newborn calves (≤30 days of age). Forty seven calves of two selected dairy herds in the province of Constantine were involved in this study. Clinical examination and analysis of blood samples collected from untreated calves over 3 periods (birth to 7 days, 8 to 15 days and over 15 days) were carried out from February to October 2013. Hematological (Hemoglobin (HGB) and Hematocrit (HCT)), biochemical (Sodium (Na^+), Potassium (K^+), Chloride (Cl^-) and pH) and organic parameters (Total protein, Glucose, Urea and Creatinine) were analyzed at the onset of diarrhea, before any treatment in diarrheic calves and subsequently compared with the values obtained in clinically healthy calves and standards reported in the literature. Base excess (BE) and the strong ions difference (SID) were also calculated by the previous assays. Results of this study showed that respiratory rate was significantly higher in calves with diarrhea (P<0.05). The occurrence of a minimal insignificant metabolic disturbance (P>0.05) was observed in diarrheic calves. A significant increase in total protein, and an insignificant difference for the rest of the analyzed hematological and biochemical parameters in diarrheic calves were observed at the statistical level P<0.05 during the experiment. A negative energy balance reflected by hypoglycemia (107.2±2.5 mg/dL in diarrheic calves versus 121.8±4.6 mg/dL in healthy calves) was also noted during the diarrheal syndrome (P>0.05).

Keywords: acidosis, dehydration, neonatal diarrhea, total protein, Algeria.

1. Introduction

Diarrhea (scours) is the most commonly reported calf disease and the major cause of neonatal mortality in both dairy and beef calves less than one month old.
It represents an important source of economic loss due to treatment costs, stunted growth rates and calf mortality (Boussena and Sfaksi 2009; Lorenz et al. 2011).
The etiology of scours is multifactorial. The known causes are grouped into two categories: non infectious causes as inadequate nutrition, and infectious causes such as bacteria, viruses and protozoa (Stoltenow and Vincent 2003). Enterotoxigenic *Escherichia coli* K99/F5 (ETEC), rotavirus A, Bovine coronavirus and *Cryptosporidium* spp. are the most common infectious agents implicated with the etiology of this syndrome (Katsoulos et al. 2017).
Regardless of the cause, diarrhea can lead to dehydration, electrolytic imbalances, metabolic acidosis and possibly septicaemia due to the secondary bacterial overgrowth in the small intestine (Navetat et al. 2007; Berchtold 2009; Taylor et al. 2017).
Therefore, it would be of great value for the success of treatment if the farmers had a quantitative indicator available to use for the early detection of the symptoms associated with these complications (Katsoulos et al. 2020).
To our knowledge, studies on metabolic defect associated with calf diarrhea have never been carried out in Algeria. The aims of this work are to study the pathogenesis of neonatal diarrhea in young calves aged less than 30 days, and to characterize the main clinical consequences and biological parameters disturbances due to diarrheal syndrome.

2. Materials and methods

2.1 Animals

This study was carried out in the region of El-Khroub in the province of Constantine northeastern Algeria, located in the semi-arid area at an altitude of 597 m above sea level, latitude 36.26° N and longitude 6.69° E. The study was performed from February to October 2013 in two selected dairy herds with no recent history of a severe scours epidemic. It should be mentioned that no prior scours vaccination was administered to cows in these farms.

A total of 47 calves (25 males and 22 females) aged less than 30 days (43 Holstein calves, 3 Tarentaise and one calf of Balzadaise breed) were selected for the experiment. During the nine months of the study period, 14 calves (29.79%) have contracted diarrhea.

Immediately after birth, the calf's navel is disinfected. The animal received 3 to 4 liters of colostrum by bottle-feeding within the first few hours after birth, and then housed individually in a single pen. Every day and for a month, calves are fed cow's milk (twice a day) at about 10% of their body weight. All experiment procedures were conducted in accordance with the animal welfare regulations.

2.2 Clinical examination and weight measurements

For each selected calf (clinically healthy or diarrheic) a thorough clinical examination is performed. Fecal samples from calves without (n=33) or with (n=14) diarrhea were examined (consistency, color, odor and presence of foreign elements).

Body weight (BW) was estimated with weight estimation formula based on heart girth (c) measurements using a common tape (±5-6% of body weight):

$$BW \ (kg) = 100 \times c^3$$

The degrees of dehydration (Guatteo 2004) and acidosis assessed according to age and clinical signs (Naylor et al. 2003; Ravary et al. 2006) were also recorded for calves during the diarrheal episode.

2.3 Blood sampling and analysis

Blood samples (5mL) were collected via jugular venipuncture using two anticoagulant tubes (EDTA, Heparin) and one plain vacuum tube. The blood samples were kept in an insulated cooler and transported to the analysis laboratory within 2 hours after collection.

The following hematological and biochemical parameters were analyzed:

- Hematocrit and hemoglobin were measured with a Mythic 18 (Orphée SA, Switzerland) automated analyser.

- Serum electrolyte levels (Na^+, K^+, and Cl^-) were analyzed by EasyLyte Electrolyte Analyzer (Medica Corporation, Netherlands).

- A spectrophotometric method was carried out for the determination of total proteins in blood plasma (Biuret method).

- Glucose, urea and creatinine were measured using a Daytona Autoanalyzer (Randox Laboratories, UK).

- Blood pH was also determined with a pH meter (pH 210, Hanna Instruments, Italy).

- Base excess (BE) was calculated using the formula reported by Nappert and Naylor (2001):

$$BE \ (mmol/L) = -301.158 + 39.617 \times pH$$

- Strong ion difference ($[SID^+]_m$) was calculated according to the formula reported by Guatteo (2004):

$$[SID^+]_m \ (mmol/L) = [Na^+] + [K^+] - [Cl^-]$$

2.4 Statistical analysis

All statistical analyzes were performed with the Statistical Analysis System software (SAS 2003) using the Student test to identify the significant difference between the calves with and without diarrhea. Data were analyzed using PROC MEANS, with health status serving as the fixed effect. P-values less than 0.05 were considered as significant. The presented results are expressed as mean and standard error of the mean (SEM).

3. Results and discussion

3.1 Clinical parameters

During diarrheal episodes, meaningful and effective changes in stools were observed. Foul smelling feces (putrid or butyric odor in 36% of fecal material) with watery or pasty like consistency in 71% of fecal material. Color of diarrheal stools varies from pale (79%) to dark yellow (21% of cases) and flecks of mucus or blood are evident in 36% of fecal samples.

Table 1 shows the mean values of body weight and physiological parameters (rectal temperature, respiratory and heart rates) expressed in their conventional units of measurement compared together with usual values and with those recorded in clinically healthy calves during the experiment.

Table 1: Average values (±standard error) of body weight and physiological parameters in diarrheic and clinically healthy calves compared with physiological norms during the first month of life

Parameters	Body weight (kg)	Respiratory rate breaths per min	Heart rate beats per min	Rectal temperature (°C)
Reference values	/	20-50 (Malheu 2007)	126±4 (Walker et al. 1998)	38.5-39.5 (Schelcher 2008)
Non diarrheic calves (n=33)	48.8±1.7[a]	49.5±2.3[a]	120.5±5.5[a]	38.7±0.1[a]
Diarrheic calves (n=14)	43.6±2.2[a]	61.1±3.2[b]	124±5.4[a]	39±0.2[a]

[a, b]: means within the same column with different superscripts differ significantly (P<0.05).

The results show that body weight, rectal temperature and heart rate in the first month of life are not influenced by diarrhea. However, an insignificant modification of these parameters was observed in diarrheic calves compared with those clinically healthy (P>0.05). Overall, a tendency was noted for the diarrheic calves to have a significantly higher respiratory rate (P<0.01) (Table 1).

In the present study, body weight did not differ significantly between healthy (48.83±9.74 kg) and diarrheic calves (43.51±9.10 kg), which is consistent with the work of Rollin (2002).

Because water represents more than 70% of the calf's body weight and the extracellular fluids accounted approximately for 45% of the newborn body weight (Thomson 1991; Berchtold 2009), any change in the fluid status is known to influence powerfully body weight (Rollin 2002). In fact, it was also noted that the newborn calves are more sensitive to liquid losses compared to adults (Thomson 1991; Berchtold 2009). The resultant weight loss in the current study was estimated at about 11%, which agrees with previous reports in the literature (Walker et al. 1998; Leal et al. 2008). However, in the study of Ferreira (2001), calves with naturally acquired diarrhea tended to have a lower weight loss.

One of the more intriguing findings of this study was the significantly higher respiratory rate in calves with diarrhea (P<0.05). The same result was reported by El-sheikh et al. (2012). This finding could be attributed to the decreased blood pH, which in turn would stimulate the respiratory center of the medulla oblongata and thereby result in an increase of respiratory rate and depth (El-sheikh et al. 2012). As mentioned by El-sheikh et al. (2012), it is a compensatory polypnea in response to acidosis leading to eliminate this CO_2 excess in order to reach normal values of pH. In another study, lower apparent respiratory rate was noted (23.46±2.71 to 25.33±4.75 breaths) (Leal et al. 2008).

Similar to the respiratory rate, diarrheic calves tented to have an increased heart rate when compared with controls (P>0.05). This observation coincides with previous reports (Walker et al. 1998; Leal et al. 2008). In fact, it appears that this tachycardia compensates the hypovolemia due to dehydration during diarrheal episodes. Previous research (El-sheikh et al. 2012) indicates that tachycardia (120-140 beats/min) was recorded at the early stage of diarrhea (with moderate dehydration) and bradycardia (between 50-88 beats/min) with cardiac arrhythmia was observed at severe dehydration.

No significant changes occurred in rectal temperature in response to diarrhea (P>0.05). This is in accordance with previous reports (Leal et al. 2008; Malik et al. 2013).

Furthermore, rectal temperature could be influenced by the duration of the diarrheal episode and the severity of dehydration (first there is an increase in temperature in early stage of diarrhea (39 to 40.5°C) and then, it decreased in severe cases of diarrhea (36 to 38°C) (El-sheikh et al. 2012). Therefore, Berchtold (2009) concluded that the decrease of rectal temperature is the logical consequence of dehydration in scouring calves.

In agreement with the result reported by Guatteo (2004), 64% of diarrheic calves had no clinical signs of dehydration. This has also been observed before by Albin (2002). Seven per cent (7%) of them required intravenous hydration (severe dehydration).

According to clinical criteria, no base deficit occurred in 73% of diarrheic calves at less than 8 days of age. Within this age group, 18% of calves with diarrhea had a base deficit value of 10mmol/L, and 9% of them had a base deficit estimated at 5mmol/L.Based on the clinical symptoms, base deficit was more prevalent in older calves. Two-third of calves with diarrhea older than 8 days had a base deficit value of 15mmol/L. No deficit (BD=0) was noted for the rest of calves of this age group.

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In both age groups, mild to moderate acidosis had occurred in 36% of scouring calves. Throughout the first month of life, the majority of calves (64%) had not shown signs of acidosis during the diarrheal episodes.

In our experiment, calves presented a very similar clinical signs of acidosis to those noted in some other reports (Berchtold 2009; Trefz et al. 2012).

3.2 Biological parameters
3.2.1 Markers of dehydration
Table 2 summarizes the results of different markers of dehydration (total proteins, hematocrit, hemoglobin, urea and creatinine) in diarrheic and clinically healthy calves compared with norms reported in the literature.

Table 2: Means and standard error of different markers of dehydration in the two studied groups (diarrheic and healthy calves) during the first month of life compared with reference values

Parameters	Hematocrit (%)	Hemoglobin (g/L)	Total Proteins (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Reference values (Guatteo 2004; Navetat and Rizet 2002; Navetat et al. 2007)	24-46	6-8	6-7.6	7-20	10-15
Non diarrheic calves (n=33)	23.8±0.9[a]	7.8±0.3[a]	6±0.3[a]	24.9±2.3[a]	14.2±1.1[a]
Diarrheic calves (n=14)	24.6±1.4[a]	7.8±0.6[a]	7.5±0.5[b]	29.5±3.5[a]	13.4±1.7[a]

[a, b]: means within the same column with different superscripts differ significantly (P<0.05).

In the current study, diarrhea elicit significant changes in total proteins concentration (P<0.05). This observation coincides with those of various authors (Walker et al. 1998; Guzelbektes et al. 2007; Leal et al. 2008; El-sheikh et al. 2012).

A possible reason for this hyperproteinemia could be the hemoconcentration phenomenon, presumably due to dehydration (Navetat and Rizet 2002; Naylor et al. 2003). As mentioned by these authors, it is also reasonable to think that total proteins can also differ according to the effectiveness of the passive transfer of colostral immunoglobulins (Navetat and Rizet 2002; Naylor et al. 2003).

The comparison of different blood parameters considered as markers of dehydration between diarrheic and normal calves showed that, hematocrit and hemoglobin were not significantly different (P>0.05) between these two groups.

Numerous authors (Guzelbektes et al. 2007; Malik et al. 2013) noticed that the no significant elevation in both hematocrit and hemoglobin levels can be attributed to the diarrheal disease. Increased but significant hematocrit was observed during scours in other studies (Walker et al. 1998; Leal et al. 2008; Freitas 2009; El-sheikh et al. 2012).

Indeed, an effect of week (age of calves) was previously observed (the concentration of hematocrit tended to be higher at birth and then decreased with age) (Klinkon and Ježek 2012).

In this study, the lack of significant differences between the two groups (diarrheic and non diarrheic calves) in hematocrit and hemoglobin levels is probably observed because calves are not analyzed at the same age (week of age) and large numbers of calves should be tested.

Hematocrit provides important information about the overall blood volume. Furthermore, hemoglobin is related to the rate of oxygen transported in the bloodstream (Malheu 2007). Indeed, hematocrit and hemoglobin accurately reflect the hydration status and used to assess it. Polycythemia results from decreased extracellular fluid volume, especially plasma, which indicates a hemoconcentration due to dehydration (Walker et al. 1998; Freitas 2009; Malik et al. 2013).

Unlike creatinine, the values of urea in our samples are higher than reference values claimed by different authors (Guatteo 2004; Navetat and Rizet 2002; Navetat et al. 2007). Since the concentration of urea in blood depends on nutrition more than that of creatinine (Malheu 2007), the difference between reference values of urea and those reported in healthy calves seems to be related to the feeding regimen. Increased concentration of urea in calves' serum indicates increased catabolism of proteins (El-sheikh et al. 2012; Klinkon and Ježek 2012).

In the present study, urea level was reported to be higher in diarrheic calves (P>0.05) when compared with healthy animals. This finding was consistent with results in previous study (Demigné and

Rémésy 1979). Similar but significant (P<0.05) increase was previously reported in calves suffering from diarrhea (Walker et al. 1998; Guzelbektes et al. 2007; Özkan et al. 2011; El-sheikh et al. 2012). According to Bouda and Jagoš (1984), increased urea levels found in calves suffering from diarrhea are indicative of the increased dietary intake of nitrogen compounds.

This increase is more likely attributed to hypovolemia due to dehydration (Fattorusso and Ritter 2006; Grech-Aneglini 2007; Freitas 2009).

It appears, therefore, that the measurement of blood urea concentration is very helpful for assessment of dehydration and disturbances of acid-base balance in calves with diarrhea.

In contrast to previous reports (Walker et al. 1998; Leal et al. 2008; El-sheikh et al. 2012), a slight decrease in creatinine (P>0.05) was noted in diarrheic calves, but still within the reference range (Guatteo 2004). Diagnostically, it is important for the assessment of functioning of the glomerular system in the kidneys (Klinkon and Ježek 2012). These authors added that its concentration increase only at serious damage. Thus, renal perfusion rate decreases as well as glomerular filtration and lead to signs of functional renal failure (Albin 2002; Gyr et al. 2004). Indeed, urea and creatinine levels are sensitive indicator of renal insufficiency (Malheu 2007).

3.2.2 Acid-base balance parameters

Hydrogen ion concentration (pH), serum chloride, potassium and sodium levels in healthy calves remained within physiological norms typical for the species. In the calves with diarrhea, disturbances in the acid-base balance manifested by decreased values of pH, Na^+ and Cl^- ions, reduced base reserves were observed, with no significant differences recorded (P>0.05) (Table 3).

Table 3: Means values of glucose, acid-base balance parameters and electrolyte levels in diarrheic and non diarrheic calves' blood compared with physiological norms during the first month of life

Parameters	Glucose mg/dL	pH	BE mmol/L	Na^+ mmol/L	Cl^- mmol/L	K^+ mmol/L	$[SID^+]_m$ mmol/L
Reference values (Guatteo 2004, Navetat and Rizet 2002, Navetat et al 2007)	55-95	7.35-7.45	-2 to +2	136-147	95-105	4-5	35-45
Non diarrheic calves (n=33)	121.8±4[a]	7.4±0.1[a]	-8±1.9[a]	131.2±0.6[a]	101±0.9[a]	5±0.1[a]	35.2±0.6[a]
Diarrheic calves (n=14)	107.2±6[a]	7.3±0.1[a]	-11.2±2.8[b]	129.7±0.9[a]	99.8±1[a]	5±0.1[a]	35±0.9[a]

[a, b]: means within the same column with different superscripts are significantly different at P<0.05

The recorded pH values in clinically healthy or diarrheic calves are in accordance with reference values (Guatteo 2004; Navetat and Rizet 2002; Navetat et al. 2007). During diarrhea, a non significant small decrease in blood pH (7.3±0.1) was noted (P>0.05), which seems to confirm the presence of a mild metabolic acidosis (Walker et al. 1998; Freitas 2009). A similar but significant decrease was previously reported by Guzelbektes et al. (2007).

Metabolic acidosis is a result of the loss of carbohydrates and also from organic acid accumulation. In a healthy organism, lactic acid is easily excreted by the kidneys. However, this excretion is impaired during dehydration (Dratwa-Chałupnik et al. 2012). It should be noted that blood pH is a simple and useful indicator of clinical health and it would be useful diagnostic and prognostic tool at the farm level (Sayers et al. 2016). It suggests, therefore, that monitoring pH alone would be useful in defining acidosis. The availability of simplified, economical pH meter, would therefore improve accurate diagnosis of acidosis during diarrheic episode.

Base excess is the most useful parameter for quantifying the acid-base balance. Its estimation is of benefit in confirming a diagnosis of metabolic acidosis and determining prognosis in diarrheic calves (DiBartola 2012).

Findings of this study indicate that even clinically healthy calves have a clear negative base excess (-8 mmol/L). However, Guatteo (2004), Navetat and Rizet (2002) and Navetat et al. (2007), indicate that base excess in healthy calves is ranged from -2 to +2. In our study, base excess was determined by using the equation of Nappert and Naylor (2001).

That equation was derived from blood pH, which itself measured by portable pH meter (twin Cardy pH meter) relatively inexpensive alternative of blood gas analyzers.

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Since all pH measurements were obtained from Hanna pH meter and pH mean values in healthy calves were in line with previously published reference ranges (Guatteo 2004; Navetat and Rizet 2002; Navetat et al. 2007). Therefore, we question the appropriateness of using the formula of Nappert and Naylor (2001) for calculating base deficit using this pH meter. Our base deficit may have been affected by the use of this equation and perhaps a more accurate equation of base excess estimation (based on the results of pH meter) should be proposed. During diarrheal episodes, laboratory analysis revealed a non significant decrease of BE (-11.2±3 mmol/L), whereas a significant decrease was recorded by various authors (Guatteo 2004; Guzelbektes et al. 2007; Freitas, 2009; Trefz et al. 2012).

Strong ion difference mean values in both healthy and diarrheic calves were within previously published reference ranges (35-45 mmol/L) (Guatteo 2004; Navetat and Rizet 2002; Navetat et al. 2007).

Similar strong ion difference mean values were recorded in both healthy and diarrheic calves. However, a statistically significant decrease was previously reported during diarrheic episodes (Guatteo 2004; Smith 2009; Stämpfli et al. 2012; DiBartola 2012). In our study, the lack of significant differences in BE and SID between healthy and diarrheic calves could be related to the size of the sample (14 diarrheic calves).

In the current study, the observed values of glucose in healthy and diarrheic calves are higher than those previously published for this species (reference ranges: 55 to 95 mg/dL) (Guatteo 2004; Navetat and Rizet 2002; Navetat et al. 2007). The timing of blood analysis relative to feeding is a possible factor to the variation in this parameter. Additionally, age of calf can also be a determining factor. Glucose is generally higher among young than adults due to the lactose intake.

The results showed also an insignificant decrease of serum glucose concentration (hypoglycemia) in neonatal diarrheic calves (P>0.05) in comparison with control group (121.8±4.6 mg/dL in healthy calves vs 107.2±2.5 mg/dL in diarrheic ones). Similar but significant hypoglycemia was noticed by several authors (Klein et al. 2002; Rollin 2002; Guzelbektes et al. 2007; Kaneko et al. 2008). This hypoglycemia is due to anorexia, poor digestion and absorption, which lead to a reduced hepatic gluconeogenesis and increased anaerobic glycolysis (Albin, 2002; Rollin 2002; Kaneko et al. 2008; Freitas 2013).

In our study, serum sodium levels obtained in calves with or without diarrhea were below reference values: 136-147 mmol/L (Guatteo 2004; Navetat and Rizet 2002; Navetat et al. 2007).

The etiology of the hyponatremia observed in our calves might be resulting from the reduction in voluntary food (milk) and water intake (Berchtold 2009; Freitas 2013).

The calves with diarrhea presented a non significant slight decrease in serum Na$^+$ concentration compared to the controls. This is in agreement with several previous studies (Walker et al. 1998; Malik et al. 2013). Similar but significant hyponatremia was reported by various authors (Rollin 2002; Guzelbektes et al. 2007; Freitas 2009; El-sheikh et al. 2012; Freitas 2013). This hyponatremia was associated with a lower resorption and losses of this electrolyte in feces, which induce dehydration by large decrease in extracellular fluid volume and a smaller increase in intracellular fluid volume (Guzelbektes et al. 2007; Smith 2009; Malik et al. 2013).

Similar changes in serum chloride levels than that associated with Na$^+$ were noted in diarrheic calves (P>0.05). A non significant hypochloraemia was also reported in some other studies (Walker et al. 1998; Dratwa-Chałupnik et al. 2012). Hypochloraemia in diarrheic calves is probably related to the loss of this ion accompanying the sodium in stool (Malik et al. 2013). It is, therefore, not surprising that serum chloride level follows sodium level because chloride was usually found in the form of sodium chloride (Navetat and Rizet 2002; El-sheikh et al. 2012). According to Walker et al. (1998), serum chloride is not changed significantly; it is often primarily an intracellular loss. However, this finding is in contrast to results published in previous studies (Albin 2002; Guzelbektes et al. 2007; Leal et al. 2008; Malik et al. 2013) who found a slight hyperchloremia during natural or experimentally induced diarrhea.

Serum chloride levels in both healthy and diarrheic calves are within reference ranges reported by Guatteo (2004), Navetat and Rizet (2002) and Navetat et al. (2007).

Serum potassium levels (K$^+$) observed in apparently healthy or diarrheic calves are equal and comparable to the higher reference range value of 5 mmol/L (Guatteo 2004; Navetat and Rizet 2002; Navetat et al. 2007).

No significant difference was observed between the potassium levels in diarrheic and non diarrheic calves, in the present study and in the study of Malik et al. (2013). Significant hyperkalemia was reported by various authors in diarrheal calves (Walker et al. 1998; Guzelbektes et al. 2007; Leal et al. 2008; El-sheikh et al. 2012).

4. Conclusion

In conclusion, the results reported here, identify a significant change in respiratory rate in calves with symptoms of diarrhea. Lower levels of glucose in diarrheic calves compared with those in the control group are characteristics of a negative energy balance.

In addition to this, total protein levels were also significantly different in diarrheic neonatal calves. While the other studied hematological and biochemical parameters were not statistically affected during the diarrheal episode. These limited changes are indicative of a minimal insignificant metabolic disturbance ($P > 0.05$) in this group of animals.

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