

TOWARDS A BETTER UNDERSTANDING OF THE RESPIRATORY DISTRESS SYNDROME IN FULL-TERM NEWBORN CALVES

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INTRODUCTION

The respiratory distress syndrome (RDS) is a well-known trouble in premature infants and other newborn mammals. Prematurity has also been associated with RDS in calves but, in the veterinary literature, few RDS descriptions concern calves born at term. Now, hundreds of mature calves showing tachypnoea in the hours following birth are observed each year in the southern part of Belgium, mainly in the Belgian Blue (BB) double-muscled breed. An incidence study performed in 50 randomly selected BB farmings revealed that nearly two thirds of them had to face RDS in some of their newborn calves and that 36% of them had to deplore one or more losses each year due to this clinical entity¹. First, a field study was designed to get more insight into this RDS by means of clinical, laboratory and necropsy observations on these newborn calves. After that, several pathogenic hypotheses were tested with the aim of effectively treating and especially preventing the disease.

CLINICAL FINDINGS

A total of 53 RDS calves ranging from 6 hours to 6 days old (32 males and 21 females, 51 BB and 2 Aquitaine Blond, 49.5 kg on average) was physically examined on the field and compared to 42 healthy BB 48-hour-old calves (17 males and 25 females, 48.0 kg on average). All calves were born at term, as confirmed by their high birth weight but also by the date of mating, insemination or transplantation of the dams that delivered exclusively by caesarean section (C-section) mostly performed after the spontaneous beginning of the parturition process, with the exception of the 2 Aquitaine Blond calves that were born by vaginal delivery.

After an asymptomatic period variably lasting between ½ to 40 hours, these double-muscled calves suddenly showed spectacular tachypnoea and tachycardia (mean of 105 breaths and 151 beats.min⁻¹), quite often after the first colostrum intake. These values are to be compared to the 63 breaths and 120 beats.min⁻¹ obtained on average in control calves, that is to say also rather high values that could however be explained by the proportionally reduced weight of their heart (- 15%) and lungs (- 19%) in comparison with Holstein Friesian calves. One of the most important anamnestic elements was the precise age of the RDS calves at beginning of symptomatology. Indeed, this data combined with information on their mental status and appetite led us to distinguish two subgroups of calves: the depressed (n = 30) and undepressed (n = 23) RDS calves. In depressed RDS calves, the general clinical assessment was rapidly affected and their appetite was decreased or even nil. They balked at standing up, developed dyspnoea in addition to tachypnoea, and sometimes presented cyanotic mucous membranes, then possibly associated with turgescient jugular veins in dying calves. Now, mortality has only been recorded in this depressed subgroup of calves (n = 11/30). Respiratory rate (RR) tended to be slightly lower and heart rate (HR) higher in depressed (mean RR = 101 and HR = 158) than in undepressed RDS calves (mean RR = 110 and HR = 144). Hyperthermia (> 39.5°C) was sometimes recorded in undepressed as well in depressed RDS calves but was far from being the general rule. RDS calves showed no abnormal nasal discharge, cough or stridor. Attentive auscultation of their lungs only revealed increased breath sounds and rarely crackles and wheezes.

Symptoms appeared significantly earlier in depressed (after 2 hours of life on average) than in undepressed (after 20 hours of life on average) RDS calves, thus giving a non negligible prognostic value to the age at symptoms appearance. Unless the treated calf previously died, the symptomatology lasted for about 5-7 days before dramatically solving without sequelae.

CLINICAL PATHOLOGY

Physical examination was supported by arterial and venous blood sampling in order to measure blood gases and pH, glycaemia and lactacidaemia on the field, using portable analysers. Haematological analysis, total protein electrophoresis, ions (Na, K) and enzymes (AST, LDH, CPK) measurements were committed to the laboratory care.

In comparison with the control group ($\text{PaO}_2 = 78.1$ mm Hg, $\text{PaCO}_2 = 46.1$ mm Hg, $\text{pHa} = 7.38$ and lactacidaemia = 2.4 mmol/L, on average), depressed RDS calves were in arterial mixed (respiratory and metabolic) acidosis, severe hypoxaemia and hypercapnia ($\text{PaO}_2 = 51.1$ mm Hg, $\text{PaCO}_2 = 56.3$ mm Hg, $\text{pHa} = 7.31$ and lactacidaemia = 4.6 mmol/L, on average) that correlated well with the observed symptomatology, the most hypoxaemic calves also being those showing cyanotic mucous membranes. Undepressed RDS calves were normocapnic ($\text{PaCO}_2 = 45.2$ mm Hg) and their degree of metabolic acidosis (lactacidaemia = 3.8 mmol/L, on average) was not sufficient to significantly influence their blood pH value ($\text{pHa} = 7.36$), even if their mean arterial oxygen partial pressure tended to be lower ($\text{PaO}_2 = 70.2$ mm Hg) than in control calves.

Despite great variability, a significantly lower glycaemia value was measured in the depressed RDS calves (70 mg/dL) in comparison with the control group (93 mg/dL). Some of these depressed RDS calves were even hypoglycaemic (< 54 mg/dL).

Compared to the values of the control group, the ions, haematocrit and red blood cells levels were unremarkable in the 2 RDS groups. On the other hand, a leukocytosis was demonstrated only in the depressed RDS group (14,200 WBC/ μl). However, considering all RDS calves individually, nearly half the calves presented a leukocytosis and a leukocytes count compatible with an infection, even if the intervention of the stress induced by the disease or by the use of therapeutical glucocorticoids cannot totally be excluded.

On the average, RDS calves had a significantly lower total serum protein content (53.0 and 55.2 g/L for depressed and undepressed RDS calves, respectively) than healthy control calves (59.1 g/L). If the value of 10 g γ -globulins/L is taken as the threshold value for an adequate passive transfer of colostral immunoglobulins, 60% of the depressed and 40% of the undepressed RDS calves showed a relative failure at this point of view.

Considering that no noteworthy increase of the muscular enzymes was registered in all but one RDS calves, it was concluded that it was not a primary myopathic problem with associated dyspnoea.

NECROPSY FINDINGS

Every time a calf died, a complete necropsy was performed in the shortest delay as possible, associated with bacteriological, virological and histopathological analysis. Blood from the heart, lung aspirate, carpal synovial fluid, mesenteric lymph node aspirate and small intestine content were aseptically sampled for bacteriological culture. Viruses (RSV, IBR, PI₃, adenovirus and torovirus) were investigated by immunofluorescence in lung pieces as well as the BVD virus in the spleen. Several lung pieces were used for histopathology. The reports of 67 necropsies performed on BB calves dead after a clinical course of RDS were also retrospectively analysed.

Pulmonary necropsy findings on all these RDS calves were a combination of atelectasis, emphysema, interstitial oedema, congestion and red hepatization. Atelectasis involved always the

apical and cardiac lung lobes and, in the most severe cases, more than half of the diaphragmatic lobes. Emphysema could be present in 2 different forms: either a multitude of small interstitial bubbles of ± 1 mm diameter localized on the entire lung, and/or greater bubbles 2-3 cm diameter localized preferentially on the back of the diaphragmatic lobes and under the pleura. Lesions found by optic microscopy on some of these collapsed lungs were various combinations of congestion, haemorrhagic intra-alveolar oedema, hyaline membranes and the infiltration of the interstitium and the airspaces mainly by mononucleated cells but also by polymorphonuclear neutrophils.

Interestingly, even though they did not present diarrhoea in their life-time, 42% of the necropsied calves also presented an acute enteritis, sometimes haemorrhagic, with associated mesenteric adenitis.

Results of the microbiological analyses performed on 48 dead bodies of RDS calves revealed a septicaemia in 15 (31%) of them, 13 due to *Escherichia coli*, 1 due to *Salmonella typhimurium* and the last due to a *Streptococcus* of the group B of Lancefield. Nineteen out of 24 intestinal content samples contained non-haemolytic *E. coli* while 4 of them were positive for *Clostridium perfringens* ($> 10^8$ CFU/ml intestinal content). When possible (only 2 opportunities), the *E. coli* strain found in the intestinal content was compared with the one isolated in the heart blood. In each of these 2 calves, the strains could not be distinguished from each other by typage or comparison of their antibiograms. All these results are also to be interpreted taking into account the high amounts of antibiotics previously administered as treatment, mainly by the intravenous route, to these valuable animals.

Concerning viruses, the lung of only one calf was positive for IBR by immunofluorescence.

ETIOPATHOGENESIS

The extended atelectasis lesions systematically found in dead RDS calves (i.e. depressed ones) led us to hypothesize a primary or secondary surfactant deficiency resulting in major pulmonary functional disturbances. Indeed, the physiological roles of surfactant are of major importance in pulmonary mechanics and include a decrease in surface tension at the air-liquid interface during lung deflation, preventing alveolar collapse and stabilising the small alveoli. It also minimizes the work required for respiration, offers a barrier to fluid transudation and is involved in the innate non-specific defence mechanisms of the lungs. RDS due to a primary quantitative surfactant deficiency has been well described in premature Holstein Friesian calves² but the problem here described is clearly not associated with prematurity.

However, primary qualitative surfactant deterioration could not be excluded. It is the reason why we analysed for composition and surface activity the pulmonary surfactant isolated from bronchoalveolar lavage fluids recovered from 14 BB newborn calves that died from RDS and from 7 healthy controls³. The surface tension properties of the samples of crude surfactant were assessed by means of a pulsating bubble surfactometer. Pulmonary surfactant consists mainly of a mixture of about 90% of lipids (mostly phospholipids) and 10% of proteins by weight. The presence of phosphatidylcholine and especially the disaturated form, dipalmitoylphosphatidylcholine, is believed to be of major importance in the surface tension lowering properties of surfactant. Four surfactant-associated proteins (SP) have also been identified. Two small hydrophobic surfactant proteins, SP-B and SP-C, are thought to be essential for the rapid adsorption of the phospholipids to the interface⁴, while two large hydrophilic surfactant proteins, SP-A and SP-D, have been implicated in defence mechanisms of the lungs and in the secretion and recycling of surfactant^{5,6}. In our study, major biochemical modifications

associated with altered static and dynamic surface tension properties were demonstrated in pulmonary surfactant isolated from the RDS calves³. In particular, alterations in the ratio of total proteins to phospholipids, the phospholipid profile, SP-A levels and especially extremely low or undetectable SP-C levels were found in these samples. This last abnormality associated with protein alveolar flooding and secondary surfactant inhibition was thought to be responsible for the death of these animals. However, the cause of the very low SP-C content is still not identified (mutation/deletion in the gene encoding pro-SP-C or inhibition of one or several steps in the intracellular enzymic processing of pro-SP-C to the mature SP-C peptide ?) and, on the other hand, surfactant from undepressed as well as depressed RDS calves that survived was not investigated.

Beside this low SP-C content, we had to consider other possible causes leading to secondary surfactant deficiency with functional defect in the hours following birth, i.e. asphyxia, acidosis, hypercapnia, septicaemia, endotoxaemia and all kinds of shock. It has been demonstrated that the C-section performed without prior traction on the calf, as soon as the dam gets prepared, considerably reduces neonatal metabolic acidosis and anoxia associated with calving, and does not impair the calf respiratory adaptation during the first 48 hours of life^{7,8}. These conclusions led us to exclude hypoxaemia, hypercapnia, acidosis, the non-resorption of fetal lung fluids and all kinds of shock as an aetiology of secondary surfactant deficiency in the RDS calves.

However, the hypothesis that the affected newborn calves suffer a septicaemia-endotoxaemia from digestive origin with various pulmonary repercussions had to be considered at the light of the necropsy findings. It was tested by measuring the endotoxin level in the blood of 14 RDS (10 depressed and 4 undepressed) and 9 healthy (4 BB and 5 Holstein Friesian) calves by the Limulus Amebocyte Lysate test. Results clearly demonstrated a higher endotoxaemia in the depressed RDS calves (0.14 Endotoxin Unit (EU)/ml) than in undepressed RDS (undetectable level) and control (0.01 EU/ml) calves. The fact that no endotoxin was detected in the undepressed RDS calves pleads for endotoxaemia being a consequence rather than the cause of the RDS. In any case, when endotoxaemia is present, the calf enters in a vicious circle, given that endotoxin suppresses surfactant synthesis by type II alveolar epithelial cells⁹ but also that the cellular inflammatory response to endotoxin includes the increase of the pulmonary arterial pressure and permeability of the pulmonary capillaries with the following disastrous consequences : oedema, crossing of proteins in the alveoli, hyaline membranes formation and alteration of the alveolar surfactant system. Hypoxia could also contribute to these effects by damaging the type II pneumocytes and by inducing vasoconstriction of pulmonary capillaries.

Lastly, seeing the lung immaturity of these full-term RDS calves, it was still legitimate to consider subclinical trace elements deficiencies in their mothers, especially iodine (I) and selenium (Se). Indeed, the thyroid hormones T3 and T4 are known to play an important role in the maturation of the surfactant system. In fact, type II alveolar epithelial cells have receptors to these thyroid hormones¹⁰ and the cellular response to stimulation by the fibroblast-pneumocyte factor, necessary for the production of surfactant of good quality, is considerably increased by T3¹¹. The effects of I deficiency are exacerbated or replaced by a lack of selenium (Se) since the deiodinase responsible for the transformation of T4 in T3 is a selenoenzyme and since T3 is ten times more active than T4¹².

More prosaically, a recent study performed in 93 beef herds distributed in the southern part of Belgium clearly revealed the importance of deficiencies in copper (Cu), zinc (Zn) and Se¹³. In a preliminary study, we have then selected 10 additional BB farms where many RDS cases occurred (morbidity and mortality rates due to RDS = 5-33% and 6-80%, respectively) and

investigated their status in Cu, Zn, Se and I in comparison with those in 7 control BB farms with no RDS cases. Plasmatic Cu and Zn concentrations and glutathion peroxidase activity in the red blood cells were measured in 99 and 70 healthy cows from the RDS and control farms, respectively. I status was evaluated by determining I content of cow's milk. An I deficiency was diagnosed when the milk I content was $< 80 \mu\text{g/L}$. All RDS farms were deficient in Cu, Zn and Se while 8 RDS farms were proved to be deficient in I. In the 2 other RDS farms, lactating cows were not deficient in I but were supplemented by a mineral complex containing I that was not distributed during pregnancy. Consequently, I deficiency could not be excluded in pregnant cows in these 2 farms. Concerning control farms, 5 of them were deficient in Cu and Zn while only 2 were deficient in I and Se. Moreover, and this fact is stronger than a Lord Mayor, no other calf has presented RDS in the 10 selected farms since the adequate trace elements supplementation of the cows during pregnancy. We deduce that the trace elements deficiencies in general and the ones in I and Se in particular might take on a dominating responsibility in the RDS pathogenesis in newborn calves.

However, other causes like the use of corn silage as the main fodder in the ration of pregnant cows and heifers have also to be taken into account, especially when it contains too much bypass starch. Indeed, such silage is very poor in proteins (now, proteins are the source of selenomethionine), vitamins, macro- and trace minerals, and provides a lot of glucose in the intestine of the mothers, so promoting their fattening and the calving of heavy calves (metabolic syndrome).

In summary, whatever the mechanisms leading to the initial surfactant inhibition, that probably are in close connection (low SP-C level, septicaemia-endotoxaemia, I and Se deficiencies, fat cow syndrome), the resulting atelectasis decreases lung compliance, increases the work of breathing and leads to a rapid and shallow respiratory pattern. The extent of the pulmonary shunt is function of the importance of the atelectatic lung area and the resulting hypoxaemia brings the newborn calf into a critical vicious circle : pulmonary hypoxic vasoconstriction and pulmonary hypertension with alveolar flooding of oedema fluid and secondary surfactant inhibition, maintenance of the foetal circulation, bad oxygenation of the tissues and metabolic acidosis. As a consequence, type II pneumocytes are injured and are not able to synthesize surfactant in adequate amount anymore. Through the description of the physiopathological mechanisms leading to respiratory failure, we recognize the clinical and necropsy symptoms described for the RDS calves.

THERAPY

The treatment that we usually apply does not comprise very expensive or priceless interventions such as intranasal oxygen insufflation and surfactant administration. Treatment with antibiotics and anti-inflammatory drugs, either steroidal or nonsteroidal, did not seem to influence to a great extent the course of the disease. On the contrary, treatment of RDS calves with laevothyroxine *per os* (Forthyron[®], Dechra, The Netherlands, $20 \mu\text{g/Kg BID}$) during 1 week gives better results. We associate it all the same to intravenous antibiotherapy (ampicilline for example) and flunixin meglumine to control the possible septicaemia-endotoxaemia, to Vitamin E and selenium IM and I *per os*, and to very good nursing practices. At this point of view, it is highly recommended to restrict the colostrum or milk amount offered to RDS calves¹⁴. Clinically, RDS-affected calves are worsening less and improve faster in case of milk restriction. Indeed, enterocytes are particularly prone to hypoxia that is physiologically more marked at the top of the intestinal villi and that is still aggravated by hypoxemia in RDS calves. That could be

related to the haemorrhagic enteritis (without visible diarrhea during their lifetime) found at necropsy in some RDS calves.

CONCLUSION

In conclusion, the RDS of full-term newborn calves is a very fine multifactorial disease that could certainly be used as an experimental model for mature babies suffering from RDS. Indeed, in human medicine, RDS is the leading cause of neonatal death throughout the industrialized world, especially in countries that are known to be I and Se deficient¹⁵. Human beings will rarely have been so closely related to cattle.

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KEYWORDS

Hyaline membrane disease, surfactant, etiopathogenesis, endotoxaemia, iodine and selenium deficiency