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# HISTOPATHOLOGIC ALTERATIONS OF LUNG TISSUE CAUSED BY HYPOXIA IN CALVES DECEASED DUE TO DYSTOCIA

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**Summary**

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Aim: Examination of lung tissue of bovine neonates deceased due to dystocia and characterisation of the lesion to improve reanimation protocols. Materials & Methods: Lung tissue samples from 37 calves, which died intra partum or up to 24 hours post natum were routinely processed for histopathological examination. Haematoxylin and eosin stain and Periodic acid Schiff's reagent were used as staining methods. Parenchyma with bronchioles and alveoli as well as the interstitium of bronchioles and alveoli were examined for signs of cell death, pneumonia, as well as the presence of corpuscular and amorphous elements. Results: Dystelectasis, congestion, meconium, amniotic fluid, hyaline membranes and the infiltration of inflammatory cells in the airways and/or the alveoli could be shown. A dependency between the migration of leukocytes and the occurrence of corpuscular and amorphous elements in relation to the location in the lungs could be identified. Clinical significance: These findings could be used to improve reanimation and neonatal treatment protocols. Based on the findings a non-steroidal anti-inflammatory treatment could be considered.

**Key words:** acidosis, foetal distress hypoxia, meconium, neonates

## INTRODUCTION

Dystocia induced hypoxia is the leading cause of death amongst neonates, due mainly to respiratory distress (Østerås *et al.,* 2007; Zerbe *et al.,* 2008; Bleul, 2009; Silva *et al.* 2009; Dutra & Banchero, 2011, Szenci, 2012, Edwards *et al.,* 2013). In cattle the peri-natum mortality varies between 2–10% internationally (Mee *et al.* 2008). A prolonged birth causes pathologic hypoxia with ensuing organ damage leading to unthriftiness and possibly death (Alonso-Spilsbury *et al,*. 2005; Taverne, 2008, Dutra & Banchero, 2011, Szenci, 2012, Bleul & Götz, 2013, Martz, 2017). This sustained perinatal hypoxia causes a shift from an aerobic to an anaerobic metabolism, leading to systemic acidosis and finally cellular deterioration. As Bleul & Götz (2013) showed in their work with calves, the longer parturition took the higher lactic acid was and the lower the blood pH. Further, tissue dam-

age can be attributed to an overproduction of free radicals followed by oxidative stress and increased secretion of pro-inflammatory cytokines (Alonso-Alconada *et al.,* 2012; Mokra & Calkovska, 2013; Martz 2017). For a successful adaptation to extra-uterine life it is essential that the lungs of the neonate are able to perform sufficient gas exchange. One of the consequences of foetal hypoxia is the aspiration of meconium contaminated amniotic fluid causing airway obstruction, aeration problems, impaired gas exchange, atelectasis or dystelectasis, pulmonary inflammation and surfactant dysfunction, resulting in meconium aspiration syndrome (MAS) and/or neonatal respiratory distress syndrome (nRDS) (Vidyasagar & Zagariya, 2008; Mokra & Mokry, 2011; Swarnam *et al.* 2012). Histological examination of lungs from neonates that experienced respiratory distress due to prolonged labour show squamous epithelial cells, meconium and inflammation as demonstrated by Lopez & Bildfell (1992) in their work with calves. Zagariya *et al.* (2000) were able to show in a model with rabbit pups that meconium triggers an increased polymorph-nuclear neutrophils (PMN) response of the lung tissue. Intrapulmonary PMN accumulation gravely increases the expression of pro-inflammatory chemotactic cytokines, phospholipase $A_2$ and levels of $PGE_2$, exacerbating inflammatory reactions of lung tissue and having a deleterious effect on alveolar cells (Zagariya *et al.,* 2000; Vidyasagar & Zagariya, 2008; Mokra & Mokry, 2011). These inflammatory reactions cause increased vascular permeability leading to pulmonary edema and proteinaceous exudative liquid to accumulate in the alveoli (Vidyasagar & Zagariya, 2008). During foetal life the lungs are a non-essential organ and are therefore excluded from the blood redistribution which occurs during extended labour, exposing them sooner and longer to an anaerobic metabolism (Martines-Burnes *et al.* 2002; Szenci, 2012).

The aim of this study was to examine histologically the alterations in lung tissue of bovine neonates born dead or deceased within 24 hours of birth which experienced dystocia:

- To assess what pathomorphological alterations to lung tissue can be observed;
- To identify if the distribution of these alterations is clustered in specific areas of the lung or if they are found diffusely throughout the lung samples.

Furthermore, to use these findings to create improved resuscitation and first response protocols for newborn calves.

## MATERIAL AND METHODS

All owners were informed and gave their consent before calves were included in the study. Organ tissue samples (n=148) were taken from 37 neonates which died intra partum or up to 24 hours post natum (p.n.) caused by nRDS and/or MAS, from cows presented in the clinic due to dystocia. In order to ensure the cells did not succumbed to the natural process of post mortem autolysis, the organs were extracted immediately after the foetus was delivered. The lung samples were always taken from approximately the same four location (left cranial & caudal lobs and right cranial & caudal lobs) and of approximately the same size to support consistency. Neonates with apparent macroscopic malformation as well as animals that showed signs of advanced autolysis due to prolonged intra uterine death were excluded. The tissue samples were fixed in buffered formalin, washed with a buffer

solution, dehydrated with alcohol and then embedded in paraffin blocks. The paraffin blocks were cut into four micrometer thick slices and analysed using a light microscope. The hoematoxylin and eosin (H&E) stained tissue slides were examined for signs of foetal dystelectasis, pulmonary injury, and the presence of corpuscular and amorphous elements and cellular infiltration in the interstitium and lumen of the bronchioles and alveoli. To better visualise meconium and amniotic fluid, a second batch of tissue sections were dyed using a Periodic acid Schiff's (PAS) reaction and counterstained with haematoxylin. Images of the evaluated elements were captured with a digital camera attached to the light microscope and stored on a computer. The histopathological alterations were examined, first at a 20 times magnification to ascertain the extent of the dystelectasis and measure the examined area. Then the samples were evaluated closer with a 100, 200 and 400 times magnification to better visualise cellular alterations. This was done using a standardized protocol for the H&E staining and a second time with a modified protocol for the PAS reaction (Castro-Ikeda *et al.,* 1998, Staribratova & Belovejdov, 2001; Najera *et al.,* 2006; Zagariya *et al.,* 2010). In the H&E stain the frequency and severity of the variables were scored either on a scale from 0 to 3; 0 representing no alterations and 3 sever alterations or binomially; 0 meaning not present and 1 meaning present (Martinez-Burnes *et al.*, 2002). Qualitative parameters were displayed in two dimensional frequency tables according to location. The statistical significance of differences between locations was verified with the exact Friedman-test using the program StatXact (Cytel Studio, 2010). Additionally, a two dimensional analysis of association between the appearance of leukocytes and the occurrence of corpuscular and amorphous elements was carried. Two dimensional frequency tables were generated and their relationship was tested with the exact-Wilcoxon-Mann-Whitney test also using the StatXact (Cytel Studio, 2010) software. In the assessment of the statistical significances a level of significance of $\alpha$=0.05 related to each target variable was used, all P-values $\leq$ 0.05 were interpreted as statistically significant.

## RESULTS

It was possible to show alterations which could lead to lung damage and consequentially unthrifty new born claves. In the H&E analyses following alterations were found. All of the animals displayed signs of fetal dystelectasis, with a significant association (P=0.008) between the occurrence and its distribution. Pulmonary injuries were depicted in varying degrees in all calve lungs. All calves (37, 100%) showed signs of cell debris in both their bronchiolar and alveolar lumen. In the bronchioles of 31 (83.8%) and the alveoli of 35 (94.6%) calves keratin could be displayed. Meconium was present in the bronchioles of 24 (64.9%) and in the alveoli of 33 (89.2%) of the examined calves. Amniotic fluid was exhibited in the bronchiolar lumen of 35 (94.6%) and in the alveoli lumen of all the neonates. Hyaline membranes could be depicted in the bronchioles of 23 (62.2%) and in the alveoli of 35 (95.6%) of the calves. In the alveolar lumen a significant relationship (P=0.04) between the occurrence and distribution of hyaline membranes could be shown. The migration of leukocytes into the lumen of bronchioles and alveoli could be demonstrated in 33 (89.2%) and 37 (100%) of the neonates respectively.

Leukocytes were found in the bronchiolar interstitium of 23 (62.2%) and in the alveoli septa of all the animals. In the PAS analysis the bronchiolar lumen of 32 (86.5%) and alveolar lumen of 37 (100%) neonates showed signs of amniotic fluid. Meconium was found in the bronchiolar lumen of 10 (27%) and in the alveolar lumen of 31 (83.8%) of the animals. A statistical significance between the different lung locations could not be found. In the two-dimensional analysis of dependency between the appearance of inflammatory cells and the occurrence of corpuscular and amorphous elements in relationship to the location in the lungs, it could be shown that in the bronchioles, a relationship existed between the occurrence of leukocytes and meconium (P=0.05), leukocytes and keratin (P=0.03), PMN and meconium (P=0.04) and PMN and cell debris (P=0.05). In the alveoli there was a dependency between the incidence of leukocytes and meconium (P=0.02), leukocytes and amniotic fluid (P=0.04) and PMN and keratin (P=0.04).

## DISCUSSION

This is one of a few comparative studies with alterations in lung tissue of neonatal bovines, who died due to a nRDS or MAS, examined histologically in several locations. The caudal lung lobes were significantly more often dystelectatic then the cranial lobes. Linke *et al.* (2013) showed that thrifty calves had a better aeration of the dorsal than the ventral lung segments. Describing just the opposite to the findings of this study, this in turn could speak for the pathology of our findings. Lopez & Bildfell (1992) and Eigenmann (1984) both discussed, in their work with calves, the presence of atelectasis, but did not extrapolate on the location of the poorly ventilated lung segments. Hyaline membranes were found significantly less frequently in the cranial lobes. This could be attributed to an increased disturbance in the microcirculation and permeability in the caudle lung lobes caused by the dystelectasis. In Schoon's (1989) work all animals displayed signs of dysfunctional microcirculation and permeability in varying degrees depending on gestational age and death p.n.. Dystelectasis in relationship to its location was statistically significant, the lack of statistical significance for the other parameters could be due to their disseminated distribution. A significant association between the appearance of inflammation (leukocytes and PMN) and meconium becomes apparent in both the bronchioles and alveoli. In the bronchioles the left cranial and the right caudal lung lobes showed a significant association. In the alveoli the left caudal lobe did. In 34% of the calves examined by Lopez & Bildfell (1992) meconium was present. They were further able to show that 22 of the analysed calves who displayed meconium and/or SEC were positive for signs of inflammation. With the presence of meconium there was a 1.5 higher chance of finding sings of inflammation. Tyler *et al.* (1978) showed that meconium aspiration increases the alveolar septa infiltration with PMN as well as the influx of hyaline material. The association between meconium and the presence of PMN speaks for cytokine and chemokine activation. This is in accordance with Swarnam *et al.* (2012) and Mokra & Calkovska (2013) who have shown that meconium can be detrimental to lung tissue, due to its chemotactic properties. Homberg (2015) showed that calves whose meconium was stained had a prolonged time to sternal recumbence (T-SE) and a reduced milk uptake. Therefore, it should be reiterated that neonates

stained with meconium at birth should be given extra attention. The increased migration of leukocytes into the lungs which could be shown in all calves leads to the conclusion that neonates that are born following dystocia should not only be closely monitored concerning their acid-base status but an anti-inflammatory treatment should be considered. The influx of PMN into the lung tissue and lumen is a consistent feature with MAS (Korhonen *et al.* 2003). Meconium plays a major role in interstitial pneumonia in the peri-natal phase as already discussed by Tyler *et al.* (1978) in the 1970s. Meconium also causes the inactivation of surfactant a major part of nRDS and MAS (Swarnam *et al.,* 2012; Mokra & Calkovska, 2013).

Another problem that arises in the reanimation of unthrifty neonates is the pathological metabolic/respiratory acidosis. The most commonly used buffer in veterinary neonatology is sodium bicarbonate, but due to its work mechanism it is an inappropriate choice (Bleul, 2009). When treating neonates born after dystocia an alternative buffer to sodium bicarbonate, such as carbicarb, should be used to balance the acidosis. Since it can be concluded that the migration of leukocytes is increased in calves experiencing dystocia, a non-steroidal anti-inflammatory drug (NSAIDs) could be considered as part of future treatment regimens. There is a need for further work in this area, as there are no studies addressing how newborns react and/or metabolise NSAIDs. Until this is done, NSAIDs should be implemented with caution.

## REFERENCES

Alonso-Alconada, D., E. Hilario, F. Álvarez & A. Álvarez, 2012. Apoptotic cell death correlates with ROS overproduction and early cytokine expression after hypoxia-ischemia in fetal lambs. *Reproductive Science* **19**, 754–763.

Alonso-Spilsbury, M., D. Mota-Rojas, D. Villanueva-Garcia, J. Martínez-Burnes, H. Orozco, R. Ramírez-Necoechea, A. L. Mayagoitia & M. E. Trujillo, 2005. Perinatal asphyxia pathophysiology in pig and human: A review. *Animal Reproduction Science,* **90**, 1–30.

Bleul, U., 2009. Respiratory distress syndrome in calves. *Veterinary Clinics of North America: Food Animal Practice* **25**, 179–193.

Bleul, U. & E. Götz, 2013. The effect of lactic acidosis on the generation and compensation of mixed respiratory-metabolic acidosis in neonatal calves. *The Veterinary Record,* **172**, 528.

Castro-Najera, J. A., J. Martinez-Burnes, D. Mota-Rojas, H. Cuevas-Reyes, A. Lopez, R. Ramirez-Necoechea, R. Gallegos-Sagredo & M. Alonso-Spilsbury, 2006. Morphological changes in the lungs of meconium-stained piglets. *Journal of Veterinary Diagnostic Investigation* **18**, 622–627.

Cytel Studio StatXact Vers. 9.0.0, 2010. Statistical Software for Exact Nonparametric Inference, *User Manual*. CYTEL Inc., Cambridge, MA 02139, U.S.A.

Dutra, F. & G. Banchero, 2011. Polwarth and Texel ewe parturition duration and its association with lamb birth asphyxia. *Journal of Animal Sciences,* **89**, 3069–3078.

Edwards, M. O., S. J. Kotecha, & S. Kotecha, 2013. Respiratory distress of the term newborn infant. *Paediatric Respiratory Reviews,* **14**, 29–37.

Eigenmann, U. J. E., H. A. Schoon, D. Jahn & E. Grunert, 1984. Neonatal respiratory distress syndrome in the calf. *The Veterinary Record,* **114**, 141–144.

Giles, B. L., H. Suliman, L. B. Mamo, C. A. Piantadosi, T. D. Oury & E. Nozik-Grayck, 2002. Prenatal hypoxia decreases lung extracellular superoxide dismutase expression and activity. *American Journal*

*of Physiology - Lung Cellular and Molecular Physiology,* **283**, 549–554.

Homberg, J., 2015. Vergleichende Untersuchung zum Einsatz verschiedener Erstbehandlungsmaßnahmen beim neonatalen Kalb unter Praxisbedingungen. Ph.D. thesis, Justus-Liebig-Universität, Gießen.

Ikeda, T., Y. Murata, E. J. Quilligan, B. H. Choi, J. T. Parer, S Doi, & S.-D Park, 1998. Physiologic and histologic changes in near-term fetal lambs exposed to asphyxia by partial umbilical cord occlusion. *American Journal of Obstetrics and Gynecology* **178**, 24–32.

Korhonen, K., H. Soukka, L. Kalkola, H. Peuravuori, H. Aho, K. Pulkki, P. Kero & P. O. Kääpä, 2003. Meconium induces only localized inflammatory lung injury in piglets. *Pediatric Research* **54**, 192–197.

Lindenskov, P. H. H., A. Castellheim, G. Aamodt, O. D. Saugstad & T. E. Mollnes, 2004. Complement activation reflects severity of meconium aspiration syndrome in newborn pigs. *Pediatric Research,* **56**, 810–817.

Linke, B., H. Bostedt & A. Richter, 2013. Computer tomographic illustration of the development of the pulmonary function in bovine neonates until the twenty-first day postnatum. *Veterinary Medicine International* **1**, 1–12.

Lopez, A. & R. Bildfell, 1992. Pulmonary inflammation associated with aspirated meconium and epithelial cells in calves. *Veterinary Pathology* **29**, 104–111.

Martinez-Burnes, J., A. Lopez, G. M. Wright, W. P. Ireland, D. W. Wadowska & G. V. Dobbin, 2002. Microscopic changes induced by the intratracheal inoculation of amniotic fluid and meconium in the lung of neonatal rats. *Histology and Histopathology,* **17**, 1067–1076.

Martz, P., P. Georgiev & A. Wehrend, 2017. Prolonged second stage labour and the consequences of hypoxia in the neonate. *Bulgarian Journal of Veterinary Medicine*, http://tru.uni-sz.bg/bjvm/P.Georgiev%20OnFirst.pdf.

Mee, J. F., D. P. Berry & A. R. Cromie, 2008. Prevalence of, and risk factors associated with, perinatal calf mortality in pasture-based Holstein-Friesian cows. In: *41st Annual American Association of Bovine Practitioners Proceedings*, **41**, 613–620.

Mokra, D. & A. Calkovska, 2013. How to overcome surfactant dysfunction in meconium aspiration syndrome? *Respiratory Physiology & Neurobiology,* **187**, 58–63.

Mokra, D. & J. Mokry, 2011. Glucocorticoids in the treatment of neonatal meconium aspiration syndrome. *European Journal of Pediatrics,* **170**, 1495–1505.

Østerås O., M.S. Gjestvang, S. Vatn, & L. Sølverød, 2007. Perinatal death in production animals in the Nordic countries – incidence and costs. *Acta Veterinaria Scandinavia,* **49**, S.14

Poulsen, K. P. & S. M. McGuirk, 2009. Respiratory disease of the bovine neonate. *Veterinary Clinics of North America: Food Animal Practice* **25**, 121–137.

Schoon, H.-A., 1989. Lungen- und Plazentareifung beim Rind in der Endphase der Gravitditaet. Untersuchung zur Pathogenese des Atemnotsyndroms und der Retentio secundinarum. Habilitationsschrift. Tieraerztliche Hochschule Hannover, Hannover.

Silva, L. C. G., C. F. Lucio, G. A. L. Veiga, J. A. Rodrigues & C. I. Vannucchi, 2009. Neonatal clinical evaluation, blood gas and radiographic assessment after normal birth, vaginal dystocia or caesarean section in dogs. *Reproduction in Domestic Animals,* **44**, 160–163.

Staribratova D. & V. Belovejdov, 2001. Fetal hypoxia and meconium in preterm deliveries and stillbirth. *Trakia Journal of Sciences,* **9**, 45–48.

Swarnam, K., A. S. Soraisham & S. Sivanandan, 2012. Advances in the management of meconium aspiration syndrome. *International Journal of Pediatrics,* DOI: 10.1155/2012/359571.

Szenci, O., 2012. Diagnosis of perinatal well-being of dairy calves. *Seria Zootehnie* **57**, 3–11.

Taverne, M. A. M., 2008. The relation between the birth process and the condition of the newborn piglet and calf. *Veterinary Research Communications,* **32**, 93–98.

Tyler, D. C., J. Murphy & F. W. Cheney, 1978. Mechanical and chemical damage to lung tissue caused by meconium aspiration. *Pediatrics,* **62**, 454–459.

Vidyasagar, D. & A. Zagariya, 2008. Studies of meconium-induced lung injury: Inflammatory cytokine expression and apoptosis. *Journal of Perinatology,* **28**, 102–107.

Zagariya, A., M. Sierzputovska, S. Navale, B. Uhal, O. Zagariya & D. Vidyasagar, 2011. Role of meconium and hypoxia in meconium aspiration-induced lung injury in neonatal rabbits. *Mediators of Inflammation* **11**, 1–6.

Zagariya, A., R. Bhat, B. Uhal, S. Navale, M. Freidine & D. Vidyasagar, 2000. Cell death and lung cell histology in meconium aspirated newborn rabbit lung. *European Journal of Pediatrics* **159**, 819–826.

Zerbe, H., D. K. Zimmermann, & A. Bendix, 2008. Früh- und Spätasphyxie beim Kalb: Diagnostik, Therapie und Prophylaxe. *Tierärztliche Praxis Großtiere,* **36**, 163–169.