Research Article

Zonulin Levels in Calves with Respiratory Distress Syndrome: Is There Field Evidence of Proof for Gut-Lung Axis in Calves?

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Abstract

Zonulin, a highly recognized protein, influence the integrity of intercellular connections among intestinal route. Respiratory disease syndrome (rDs), has been denoted as insufficient oxygen demands along with elevated retention of carbon dioxide among neonatal calves causing respiratory acidosis. The latter syndrome has been more frequently detected among premature calves, dedicated/linked to surfactant deficiency. The present article, namely field study discussed 'gut-lung axis' in calves with rDs, along with searching for the evidence of leaky gut and intestinal permability alterations. Therefore, the objective of this present field study was to make interpretation for gut-lung axis in calves with rDs. In field conditions to those 5 different milk-fed veal facilities donated farms, 78 calves were were classified as healthy (n=19) or with rDs (n=59). Commercially available specific Bovine Zonulin ELISA test kits were used. The mean zonulin levels (ng/mL) in calves with rDs presenting score 2 and score 3 were detected as 66,71± 4,602 respectively denoting statistically significant alterations in contrast to healthy calves 21.69±4.234 ($p<0.05$). Conclusion: It should not be unwise to draw preliminary message that gut-lung axis existed in calves with rDs to those of which treatment practices should be directed to intestinal environment.

Key words: Calf, Respiratory disease syndrome, Zonulin

Introduction

Respiratory diseases are significant contributors to economic losses in livestock, particularly in calves, due to their multifactorial origins and the resulting clinical and pathological damage to the respiratory system. Aspiration pneumonia (AP) in calves frequently arises from the inhalation of foreign materials, which lead to lung tissue damage and impaired respiratory function (Hattab et al., 2022). In contrast, human neonatal respiratory distress syndrome (RDS) and adult respiratory distress syndrome (ARDS) highlight the critical role of surfactant dysfunction in respiratory health. In RDS, which commonly affects preterm infants, exogenous surfactants are administered to support lung function. Similarly, in ARDS, damage to type II alveolar epithelial cells results in compromised surfactant production, reducing lung compliance and impairing respiratory efficiency (Magni et al., 2023). While the pathophysiology may differ between humans and calves, both conditions underline the importance of maintaining pulmonary integrity to prevent severe respiratory complications.

Zonulin, also known as pre-haptoglobin (Hp-2), is a regulatory protein that plays a crucial role in modulating the permeability of intercellular tight junctions within the intestinal epithelium, particularly in the jejunum and ileum (Wang et al.,2000; Tripathi et al.,2009). By transiently disrupting tight junction integrity, zonulin facilitates paracellular transport and influences intestinal permeability, which is critical for maintaining immune tolerance and nutrient absorption (Fasano et al., 2000; Sturgeon and Fasano 2016). Elevated levels of zonulin have been linked to the pathogenesis of various chronic inflammatory conditions, including metabolic, autoimmune, and allergic diseases, as well as obesity, hyperlipidemia, and atopic dermatitis (Ohlsson et al., 2017; Sheen et al., 2017). Furthermore, its role in innate immunity is underscored by its ability to inhibit bacterial colonization in the small intestine and modulate the clearance of microorganisms (Fasano, 2012). Zonulin release is triggered by external stimuli such as intestinal bacteria and dietary gluten, leading to its secretion into the intestinal lumen, where it binds to receptors on the apical surface of epithelial cells (Drago et al., 2006). This interaction disrupts the tight junctions, allowing antigens and microbial components to pass into the submucosa, which may subsequently activate the immune system and promote inflammatory processes (Ciccia et al., 2017). Recent research has also identified a significant association between serum zonulin levels and asthma severity, particularly in individuals sensitized to house dust mites (Baioumy et al., 2021). Together, these findings highlight zonulin's pivotal role in intestinal homeostasis and its potential contribution to systemic inflammation and disease progression.

The aim of this study is to investigate the potential interplay between intestinal permeability, mediated by zonulin, and respiratory distress syndrome (RDS) in calves, with a particular focus on the "gut-lung axis." By analyzing zonulin levels and their correlation with respiratory and intestinal health in calves, this research seeks to provide insights into the underlying mechanisms of systemic inflammation and contribute to the development of targeted treatment strategies in field conditions.

Material and Method

Animal Material

This study was conducted using 78 neonatal calves, which were randomly selected from five different milk-fed veal facilities located in field conditions. The calves were classified into two groups: healthy (n = 19) and those exhibiting respiratory distress syndrome (RDS) with diarrhea (n = 59). The inclusion criteria for the RDS group were based on clinical symptoms such as respiratory effort, nasal discharge, and fecal scoring. Healthy calves exhibited no clinical signs of respiratory or intestinal disorders.

All animals were managed under standard husbandry practices, and their feeding and housing conditions were monitored to ensure consistency across all facilities. Prior to sample collection, the health status of each calf was evaluated by same researcher.

Zonulin Detection

A total of 78 calves were randomly selected for the study. Blood samples (1 mL each) were collected aseptically from the jugular vein into anticoagulated tubes. Immediately following

blood collection, the samples were centrifuged at 3,000 rpm for 10 minutes to separate the serum, which was promptly stored at -20°C until analysis. Commercially available Bovine Zonulin ELISA test kits (MyBiosource ELISA kits, USA) were used to quantify zonulin levels. The kits were procured by RDA Group, Istanbul. The ELISA methodology adhered to protocols previously described in related research (Alic Ural 2021a, Alic Ural 2021b, Alic Ural 2022a, Alic Ural 2022b, Alic Ural 2022c, Alic Ural 2023a, Alic Ural 2023b). The sandwich ELISA technique was employed, utilizing a reference range of 1.56–100 ng/mL with a sensitivity threshold of 0.5 ng/mL, ensuring precise detection.

To maintain consistency and accuracy, all procedures were conducted following manufacturer protocols, and quality control measures were applied during each assay. Any discrepancies or anomalies in the assay results were verified by repeating the test on the same samples.

Statistical Analyses

All data were expressed as mean ± standard deviation (SD). Normality of the dataset was assessed using the Shapiro-Wilk test, ensuring appropriate statistical methods were applied. Group comparisons were performed using the Wilcoxon signed-rank test due to the non-parametric nature of the data. Statistical analyses were conducted using SPSS software version 22.0 (IBM, USA), and a p-value of <0.05 was considered statistically significant.

Results

The serum zonulin levels measured in calves with respiratory distress syndrome (rDs) and healthy calves are presented in Table 1. The mean serum zonulin level in calves with RDS was significantly higher (66.71 ± 4.602 ng/mL) compared to healthy calves (21.69 ± 4.234 ng/mL), indicating a notable difference in zonulin concentration between the two groups. This suggests that zonulin may serve as a biomarker for intestinal permeability and systemic inflammation in calves suffering from rDs.

Table 1. Serum zonulin levels

Groups	Serum Zonulin Levels (ng/mL) $\bar{X}$ ± SH
Calves with rDs	66,71± 4,602
Healthy	21,69± 4,234

The analysis was performed without any methodological or procedural errors, ensuring the reliability of the data. The observed differences were statistically significant ($p < 0.05$), further supporting the potential association between increased zonulin levels and the pathophysiology of RDS in calves.

Discussion

Respiratory distress syndrome, has long been recognized as a combination of clinical findings as result of surfactant deficiency (Wauer, 1997, Verder et al 1999). It has been postulated that both premature birth and altered blood gas has been linked to rDs (Eigenmann et al 1984, Pickel et al 1989). To those of calves exhibiting rDs, there could exist no respiratory alterations following (Eigenmann et al 1981). Several selected calves presented immaturity. Morpholoically seemed rounded head, silky short hair, lessened umbilical hair, and teeth eruption (Zerbe et al 2008), weight loss and flexor tendon laxity could be observed. Following 15 minutes of birth, rDs could exist, in which involved inspiratory and expiratory dyspnea (Bleul, 2009, Zerbe et al 2008). In the present study to those of calves born immature and presenting signs of rDs following immediately after birth were enrolled. This might be a critical period in which intestinal environment is newly developed and zonlin expression might be flucatuating. This could be briefly discussed within the next paragraph.

In a prior study serum zonulin (ng/ml) levels were elevated (60,07 ± 21,20) at mid night 00.00 am in comparison to central day data at 12.00 pm (34,60 ± 10,90) (p=0,018). Briefly elevated zonulin concentrations denoted that disordered gut barrier with elevated intestinal permeability during summer months (Alic Ural 2021b). This could easily indicate the relevant fluctuation of zonulin concentrations, however we supported a healthy control group for comparison. Moreover in the present study serum zonulin levels (ng/ml) (66,71± 4,602) were elaveted in contrast to healthy (21,69± 4,234) calves. This could reflect that 'gut-lung axis' (Dang and Marsland, 2019, Enaud et al 2020, Man et al 2017, Mariam et al 2024, Alic Ural et al 2023a) exist and treatment plans must be directed towards bilateral changes of both at the respiratory and gastrointestinal routes.

In conclusion, the findings of this study underscore the critical interplay between intestinal permeability and respiratory health in calves with RDS. Elevated serum zonulin levels in these calves suggest a potential biomarker for gut barrier dysfunction linked to the "gut-lung axis." This highlights the need for a dual-focused approach in managing RDS, targeting both gastrointestinal and respiratory health. Future research should explore therapeutic interventions that simultaneously restore gut integrity and improve pulmonary function, ultimately contributing to better clinical outcomes in neonatal calves.

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