

# Clinical, laboratory, and microbiological evaluation of neonatal calves with and without omphalopathies

## Avaliação clínica, laboratorial e microbiológica de bezerros neonatos com e sem onfalite

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### Abstract

Omphalopathies are important causes of morbidity and mortality in neonatal calves, with significant economic and zootechnical impact. The aim of this study was to characterize the clinical, laboratory, and microbiological alterations in calves with different degrees of umbilical involvement, using an adapted scoring system. Twenty calves up to 15 days of age, originating from four farms, were evaluated and classified into three groups: healthy ( $\leq 4$  points), inconclusive (5-7 points), and diseased ( $> 7$  points). The animals underwent physical examination, complete blood count, serum %Brix assessment, and collection of umbilical swabs for bacterial isolation. Significant differences were observed in neutrophil count, neutrophil-lymphocyte ratio (NLR), and rectal temperature among groups, with higher values in diseased calves. Bacterial isolation revealed a predominance of *Escherichia coli*, highlighting the opportunistic nature of this agent. The findings suggest that the NLR, in association with rectal temperature and neutrophil count, is a useful tool for the early identification of omphalopathies in calves.

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**Keywords:** Rectal temperature. Neutrophil-lymphocyte ratio. *Escherichia coli*. Cattle.

### Resumo

As onfalopatias representam importantes causas de morbidade e mortalidade em bezerros neonatos, com impacto econômico e zootécnico significativo. O objetivo deste estudo foi caracterizar as alterações clínicas, laboratoriais e microbiológicas em bezerros com diferentes graus de acometimento umbilical, utilizando escore de classificação adaptado. Foram avaliados 20 bezerros com até 15 dias de idade, provenientes de quatro propriedades rurais, classificados em três grupos: saudável ( $\leq 4$  pontos), indeterminado (5-7 pontos) e doente ( $> 7$  pontos). Os animais foram submetidos ao exame físico, hemograma, avaliação do %Brix sérico e isolamento bacteriano da região do ônфalo. Observou-se diferença significativa na contagem de neutrófilos, na relação neutrófilo:linfócito (RNL) e na temperatura retal entre os grupos, com valores mais elevados nos bezerros doentes. O isolamento bacteriano revelou predominância de *Escherichia coli*, indicando o caráter oportunista

deste agente. Os achados sugerem que a RNL, associada à temperatura retal e à contagem de neutrófilos, constitui ferramenta útil para a identificação precoce de onfalopatias em bezerros.

**Palavras-chave:** Temperatura retal. Relação neutrófilo: linfócito. Escherichia coli. Bovinos.

## Introduction

Bovine husbandry is a major component of the Brazilian agribusiness industry due to significant contribution to the country's economic growth and workforce expansion (Luz and Fochezatto, 2023; Rangel, 2025). To achieve a high-quality end-product, improvements in the pre-weaning stage/cow-calf operations for both beef and dairy systems signals the importance of proper calf raising (Danyer et al., 2024). The neonatal period is associated with the important mortality rates in cattle, with diarrhea, bronchopneumonia, and omphalopathies being the most prevalent conditions (Marcé et al., 2010; Ramos et al., 2025).

Omphalopathies are clinically relevant, as they affect umbilical structures (urachus, umbilical arteries, and umbilical vein) and can progress to systemic infections resulted from pathogen dissemination to other organs (Hathaway et al., 1993; Baird, 2016; Yanmaz et al., 2017). Multiple factors (e.g. farm location, production system, breed) affect the prevalence of omphalopathies, that can range from 3.83 to 32.3% (Bombardelli et al., 2021; Dachrodt et al., 2021; Gomes et al., 2021; Meier et al., 2024; Perrot et al., 2024). Inadequate management and insufficiently trained personnel further increase morbidity, particularly during the first three weeks of life (Oliveira Filho et al., 2007; Machado and Ballou, 2022). Early diagnosis is essential to implement preventative strategies and maintain calf health and productive performance (Meier et al., 2024). The swelling, heat, and sensitivity of the umbilical region, accompanied by fever, lethargy, and apathy, are the main clinical signs observed in calves with omphalopathies (Constable et al. 2017).

This study aimed to evaluate the use of an adapted umbilical disorder scoring system to identify pre-weaned calves presenting omphalopathies and

further characterize the clinical, hematologic, and microbiological findings from those animals to confirm their health condition, assess which parameters could contribute to a more accurate diagnosis, and evaluate the source of the umbilical infections to guide future prophylactic methods.

## Material and methods

This study was approved by the Animal Experimentation Ethics Committee of the Faculdade Santa Bárbara (FAESB) (protocol 001/2024). Twenty calves from four farms, up to 15 days post-partum, with no clinical alterations in other organ systems at the time of sampling, were included. Calves were classified into three groups according to a proposed umbilical disorder scoring system detailed in the following section. The groups were 'healthy' (score  $\leq 4$ ), 'inconclusive' (score 5-7), and 'diseased' (score  $> 7$ ).

### Epidemiological survey and physical examination

At the time of sample collection, an epidemiological survey collected the following information from the participating farms: location, farm purpose, production system, cattle breeds, neonatal management practices (umbilical care, colostrum intake, etc.), feeding methods, environmental hygiene, and general sanitary management. These aspects were used to classify the level of management as low, medium, or high.

For each calf, the following information was recorded: sex, breed, date of birth/age, calving information (natural, assisted, or cesarean), and previous disease history. All calves underwent a physical examination that included evaluation of consciousness level, heart rate, respiratory rate, capillary refill time, mucosal color and moisture, degree of dehydration, and rectal temperature.

### Clinical laboratory evaluation

Whole blood was collected in EDTA tubes (BD Vacutainer Precision Glide, Becton Dickinson Co., Franklin Lakes, NJ, USA) for a complete blood count via MEK-6500 automatic analyzer (Nihon Kohden, Shinjuku-ku, Tokyo, Japan). Differential white blood cell counts in 100 cells were performed manually by

evaluating the blood smear, stained with Panótico Rápido®, (Laborclin, Vargem Grande, PR, Brazil), under optical microscopy using an objective immersion lens (1000× magnification). The neutrophil-lymphocyte ratio (NLR) was calculated for each calf (Samuels et al., 2024). The determination of total plasma protein (TPP) was performed manually via a portable refractometer and fibrinogen by heat precipitation technique.

Blood samples without anticoagulant were also collected, stored at 2 to 6°C until centrifugation at 1,500 × g for 15 minutes to separate the serum. Serum was collected and stored at -20°C until analysis. Samples were thawed at room temperature for % Brix serum analysis, determined using a standard refractometer (Kasvi, Curitiba, PR, Brazil).

### **Microbiological evaluation**

Charcoal cotton swabs (Absorve®, CRAL, Cotia, SP, Brazil) were used to collect samples from the umbilical stump or cord. In calves up to three days of age, sampling was performed directly from the umbilical cord, which was still moist at this stage. In calves aged 4 to 15 days, samples were collected from the umbilical scar, inserting the swab into the dried stump cavity. When secretions were present, these were also sampled. Prior to sampling, the umbilical region was cleaned with 70% alcohol, and care was taken to avoid contact of the swab with adjacent areas.

The swabs were then inoculated into Brain Heart Infusion broth (Neogen Corporation, Lansing, MI, USA) and incubated at 37°C for 24-48 hours. Subsequently, samples were cultured on base agar supplemented with 5% defibrinated sheep blood and on MacConkey agar (Neogen Corporation, Lansing, MI, USA) for 24-48 hours. Bacterial identification was based on colony macroscopic characteristics, microscopic observations (morphology and Gram staining), and biochemical tests, which were performed using a commercial kit for Enterobacteriaceae identification (Kit Enterobacterias®, LaborClin, Pinhais, PR, Brazil).

### **Omphalopathies scoring system**

For the assessment of the omphalopathies scoring system (Supplementary material), previously described scores were adapted (Steerforth and Van

Windén, 2018; Carvalho et al., 2022). The thickness of umbilical structures was evaluated by external palpation, classified as normal (0), mild (1), moderate (2), or intense thickening (3); and by internal palpation, classified as normal (0) or thickened (1). Other parameters included pain during palpation (absent = 0, present = 1), presence of secretion (absent = 0, sanguineous = 1, purulent = 2), presence of umbilical hernia (not observed/palpable = 0, detected by palpation = 1, observed on inspection = 2), involvement of the umbilical structures (arteries, vein and urachus; none = 0, one structure = 1, two structures = 2), and systemic alterations (absent = 0, present = 1). The total score ranged from 0 to 14.

### **Statistical analysis**

Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA). Comparisons between groups (with and without omphalopathies) were performed using the Fisher test for categorical variables from physical examination and laboratory parameters. Continuous variables were assessed for normality using the Sha-piro-Wilk and D'Agostino-Pearson tests. Normally distributed data were compared between groups using Student's t-test, while non-parametric data were analyzed using the ANOVA and Kruskal-Wallis. Results are expressed as mean ± standard deviation (SD). A significance level of 5% ( $p < 0.05$ ) was adopted.

### **Results**

A total of 20 calves from four farms located in three different municipalities (Botucatu, Avaré, and Tatuí) were included (Table 1). In all calves included in the study, no clinical alterations were observed in consciousness level, capillary refill time, mucous membrane color and moisture, or degree of dehydration.

Of these, 13/20 (65%) were classified as healthy (score 0-4), 3/20 (15%) as inconclusive (score 5-7), and 4/20 (20%) as diseased (score >7). The results of rectal temperature (°C) ( $p = 0.004$ ), with higher values recorded in diseased calves compared to normal calves for all these variables. The mean ± SD of hematological parameters for the three groups is presented in Table 2.

**Table 1** - Epidemiological data of the four properties included in the current study

Farm	City	Sampled calves	Purpose	System	Breeds	Management <sup>1</sup>	Mortality <sup>2</sup>
1	Boituva	3	Beef	Intensive <sup>3</sup>	Wagyu	Low	Yes
2	Botucatu	6	Dairy	Intensive <sup>3</sup>	Holstein and Girolando	Medium	Yes
3	Botucatu	3	Dairy	Extensive <sup>4</sup>	Holstein and Girolando	Low	No
4	Avaré	8	Dairy	Intensive <sup>3</sup>	Holstein and Girolando	High	Yes

Note: <sup>1</sup>The level of management/husbandry was defined based on the cleanliness of facilities, adoption of sanitary practices (for cows and calves), control of synanthropic animals, and the breeding system adopted. <sup>2</sup>Prevalence of at least one calf death within six months from when the study was conducted. <sup>3</sup>Intensive systems were those where a high population density and confinement of animals were observed. <sup>4</sup>Extensive systems were those in which the calves spent part of the day in pasture, in contact with cows, without nutritional supplementation.

**Table 2** - Hematological parameters, serum BRIX, and clinical variables of calves classified based on the adapted umbilical disease scoring

Variable	Healthy	Inconclusive	Diseased	p-value
Red blood cells ( $10^6/\mu\text{L}$ )	$10.1 \pm 5.8$	$8.8 \pm 3.5$	$13.8 \pm 8.8$	> 0.050
Hemoglobin (g/dL)	$8.9 \pm 2.0$	$8.60 \pm 1.1$	$8.6 \pm 1.4$	> 0.050
Pack cell volume (%)	$32.9 \pm 6.7$	$32.2 \pm 4.0$	$33.0 \pm 4.2$	> 0.050
Mean corpuscular volume (fL)	$40.8 \pm 4.1$	$43.0 \pm 2.0$	$37.8 \pm 3.0$	> 0.050
MCHC (g/dL)	$26.8 \pm 2.0$	$26.5 \pm 0.8$	$26.3 \pm 1.1$	> 0.050
Red cell distribution width (%)	$16.8 \pm 2.6$	$16.3 \pm 2.1$	$17.2 \pm 1.5$	> 0.050
Total plasma protein (g/dL)	$6.4 \pm 1.1$	$6.8 \pm 0.7$	$6.9 \pm 0.4$	> 0.050
Fibrinogen (mg/dL)	$491.0 \pm 378.0$	$8.1 \pm 3.9$	$375.0 \pm 250.0$	> 0.050
White blood cells ( $10^6/\mu\text{L}$ )	$9.2 \pm 1.9$	$8.1 \pm 3.9$	$11.3 \pm 3.1$	> 0.050
Segmented neutrophils ( $/\mu\text{L}$ )	$3,572.0 \pm 1,269.0^b$	$4,229.0 \pm 2,352.0^{ab}$	$7,112.0 \pm 2,565.0^a$	0.002
Lymphocytes ( $/\mu\text{L}$ )	$4,569.0 \pm 2,184.0$	$3,046.0 \pm 1,642.0$	$2,732.0 \pm 2,214.0$	> 0.050
Monocytes ( $/\mu\text{L}$ )	$997.0 \pm 512.0$	$818.0 \pm 339.0$	$1,168.0 \pm 662.0$	> 0.050
Eosinophils ( $/\mu\text{L}$ )	$16.0 \pm 37.0$	$27.0 \pm 37.0$	$231.0 \pm 370.0$	> 0.050
Platelets ( $/\mu\text{L}$ )	$582.0 \pm 240.0$	$431.0 \pm 237.0$	$464.0 \pm 200.0$	> 0.050
Neutrophil:lymphocyte ratio	$1.1 \pm 0.8^b$	$1.6 \pm 0.7^b$	$3.7 \pm 2.2^a$	0.004
Brix refractometry ( $^{\circ}\text{Brix}$ )	$8.5 \pm 1.1$	$8.8 \pm 0.9$	$9.1 \pm 0.6$	> 0.050
Temperature ( $^{\circ}\text{C}$ )	$38.7 \pm 0.3^b$	$39.4 \pm 0.5^a$	$39.4 \pm 0.3^a$	0.004
Heart rate (bpm)	$133.1 \pm 33.4$	$152.8 \pm 29.6$	$132.0 \pm 29.0$	> 0.050
Respiratory frequency (bpm)	$57.8 \pm 15.4$	$72.8 \pm 25.2$	$47.5 \pm 18.7$	> 0.050

Note: Different letters in the same row indicate statistically significant differences between groups ( $p < 0.05$ ). MCHC = mean corpuscular hemoglobin concentration; bpm = breaths per minute.

Statistically significant differences were observed in neutrophil count ( $p = 0.002$ ) and NLR ( $p = 0.004$ ), with higher values recorded in diseased and inconclusive calves compared to healthy calves for these variables.

Bacterial agents were isolated in 50% (10/20) of the samples. *Escherichia coli* was the predominant agent identified (8/10; 80%), followed by *Proteus* sp., *Klebsiella* sp. and *Staphylococcus* spp., each detected in 1/10 (10%) of the cases (Table 3).

**Table 3** - Individual calf umbilical disorder score, farm of origin, age in days group classification, and isolated bacterial agents

ID	Score	Farm	Age (days)	Group	Isolation
1	0	1	7	Healthy	<i>Klebsiella</i> spp.
2	1	1	3	Healthy	<i>Escherichia coli</i>
6	1	2	1	Healthy	-
7	1	2	2	Healthy	-
3	2	1	1	Healthy	<i>Escherichia coli</i>
8	2	2	8	Healthy	-
10	2	2	7	Healthy	<i>Escherichia coli</i>
12	2	3	7	Healthy	-
13	2	3	8	Healthy	-
14	2	4	10	Healthy	-
20	2	4	12	Healthy	<i>Escherichia coli</i>
4	3	2	8	Healthy	-
15	4	4	5	Healthy	-
5	5	2	2	Inconclusive	-
16	5	4	3	Inconclusive	<i>Escherichia coli</i>
17	6	4	8	Inconclusive	<i>Escherichia coli</i>
11	7	3	3	Diseased	<i>Proteus</i> spp. and <i>Staphylococcus</i> spp.
18	7	4	4	Diseased	<i>Escherichia coli</i>
9	9	2	9	Diseased	-
19	10	4	8	Diseased	<i>Escherichia coli</i>

Regarding distribution by group, bacterial agents were identified in 38% (5/13) of healthy calves, 67% (2/3) of inconclusive calves, and 75% (3/4) of diseased calves. No statistically significant difference was observed in bacterial isolation among the different groups.

## Discussion

Appropriate care for calves during the post-partum and pre-weaning stages can decrease the incidence of neonatal disorders, including omphalopathies, thus containing economic losses brought by calf mortality, delayed development and veterinary services (Demir et al., 2025). Non-laborious tasks, e.g., the use of an umbilical disorder scoring system (Steerforth and Van Winden, 2018; Carvalho et al., 2022), can provide a quick and simple method to identify calves at risk or already presenting omphalopathies, for further care and resolution. Utilizing the adapted umbilical disorder scoring system and the cutoffs previously proposed by Steerforth and Van Winden (2018), it was possible to establish a clear

distinction between healthy (13/20) and diseased (4/20) calves from four farms.

Three of the 20 animals included in this study were classified as inconclusive, possibly indicating the existence of animals in recovery or in early stages of omphalopathies, conditions that still warrant further attention from the farm personnel. In such cases, a complete physical evaluation can better guide subsequent steps; however, higher sensitivity complementary examination, including hematological exam and ultrasonography of the umbilical structures, can better assess the calf's condition and detect alterations unnoticeable to the common physical examination (Guerri et al., 2020; Monteiro et al., 2022).

In the present study, rectal temperature was the only relevant marker in the general physical evaluation differentiating healthy calves from diseased and inconclusive calves, with the later groups showing higher rectal temperatures. However, cautious interpretation is necessary since fever in calves can result from multiple common pathological conditions for that age, including diarrhea, bronchopneumonia, and hemoparasitic diseases (Garcia et al., 2022; Teixeira et al., 2022; Andrade et al., 2025).

Therefore, when used in conjunction with the adapted umbilical disorder scoring evaluation, it becomes a valuable tool to identify calves presenting or at risk of omphalopathies. It is important to note that the rectal temperature in the diseased ( $39.4 \pm 0.3^\circ\text{C}$ ) and inconclusive calves ( $39.4 \pm 0.5^\circ\text{C}$ ) included in our study remained within the reference range described for their species and age (Constable et al., 2017).

The °Brix values observed in the three groups are above the established cutoff point (greater than 8.5). Brix is considered an indirect indicator of serum IgG concentration (Hernandez et al., 2016; Buczinski et al., 2017; Lombard et al., 2020), as are the TPP values, which also suggest adequate transfer of passive immunity (Feitosa et al., 2001, 2010). Efficient colostrum intake ensures appropriate antibody levels and contributes to the prevention of several neonatal diseases, including omphalopathies (Hue et al., 2021). However, despite the results obtained, it is important to emphasize that the direct determination of IgG concentration remains the standard for diagnosing failure of passive immunity transfer (Lombard et al., 2020). Calves with omphalopathies, with no progression to systemic disease, generally do not present alterations in the red blood cell component of the hematologic analysis (Salci et al., 2017), as observed in our results. However, in ruminants, neutrophils play a central role in infectious/inflammatory processes (Arfuso et al., 2023). In omphalopathies, neutrophil count increase is possibly due to neutrophil mobilization associated with local damage to the umbilical region and/or systemic response to the injury (Shecaira et al., 2025). The diseased calves included in this study presented elevated neutrophil counts compared to the healthy calves ( $p < 0.05$ ) and compared to values reported in the literature for calves at 0-30 days of age (Baccili et al., 2018), while the other white blood cell counts were not different among groups.

The NLR differed significantly among the evaluated groups, being higher in calves with omphalopathies compared to the clinically healthy calves. In neonatal calves, the NLR generally remain above 1.0 during the first week of life, followed by a decrease to values below 1.0 as they age (Baccili et al., 2018; Aydin, 2024). Similar NLR ( $2.75 \pm 0.89$ ) have been reported in calves with diarrhea caused by *E. coli*,

while other etiologies of neonatal diarrhea were associated with NLR close to 1.0 (Aydin, 2024), likely reflecting differences in the degree of tissue damage observed in these conditions.

The increased neutrophil counts ( $7,112 \pm 2,565/\mu\text{L}$ ), as neutrophils play a central role in inflammatory processes in ruminants (Arfuso et al., 2023). Therefore, the development of alterations in the umbilical region and/or associated systemic repercussions may have promoted greater tissue damage and consequent neutrophil mobilization (Shecaira et al., 2025). Omphalopathies can have an infectious or congenital etiology, with infectious omphalitis, umbilical hernia, or both encompassing the majority of the omphalopathies in calves (Hopker, 2014; Torkaman et al., 2025; ). Due to the infectious aspect of umbilical disorder, microbiological evaluation could inform the proper treatment and preventative methods to contain such condition.

Bacterial isolation from the swab of the umbilical stump from the 20 calves included in this study demonstrated a predominance of *E. coli* in all groups (healthy: 30.7%; inconclusive: 67%; diseased: 50%). This was consistent with previous studies in calves (Salci et al., 2017), foals (Rampacci et al., 2017) and humans (Yohannes et al., 2025) with omphalopathies. *E. coli* is one of various causative agents of neonatal diarrhea in calves (Cho and Yoon, 2014), and its dissemination in the environment through diarrheic animals may facilitate environmental spread under inadequate sanitary practices (Meier et al., 2024). The detection of this agent in the umbilical stump of calves from all groups highlights the importance of maintaining good sanitary practices as a prophylactic method against omphalopathies (Meier et al., 2024; Perrot et al., 2024).

Despite the small sample size, particularly for calves with umbilical alterations, the lack of assessment of passive immunity transfer, and the absence of anaerobic bacterial isolation, this study still offers relevant evidence that the use of an umbilical disorder scoring system and adequate sanitary practices could be useful for the containment of omphalopathies. Although antimicrobial susceptibility testing was not performed, those results could also provide valuable information about antibiotic selection and the presence of resistant agents, themes that were outside of the scope of this study.

## Conclusion

The combined use of umbilical disorder scoring and rectal temperature assessment on neonatal calves can assist with the identification of animals at risk or presenting omphalopathies. For more precise diagnostics, neutrophil count and neutrophil-to-lymphocyte ratio are effective parameters to separate healthy calves from calves at risk or presenting omphalopathies. The main bacterial agent isolated from umbilical stumps was *E. coli*.

## Authors' contributions

RMB and FMC were responsible for the study conceptualization. LAM, for visualization. LAM and FMC, for resources. JAAR, LAM, RMB and FMC contributed to the methodology and investigation. JAAR and RMB, to data curation. PNB and LGCS, to formal analysis. PNB, LGCS, RMB and MFC, to the study validation. FMC was responsible for the project administration, and RMB for funding acquisition and supervision. JAAR and FMC wrote the original draft, while LAM, PNB, LGCS and RMB reviewed and edited it.

## Data availability statement

Supporting data is available at the following link: <https://periodicos.pucpr.br/cienciaanimal/article/view/33600>.

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