Respiratory moisture loss in neonatal calves and their association with airway inflammation

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Abstract. In 85 Holstein calves aged 2-28 days, indicators characterizing respiratory moisture loss (RML) and their association with airway inflammation were studied. Animals were evaluated by clinical scoring system WI. 66 calves had a WI clinical score of more than 3 points or less (healthy), 19 more than 3 points (sick). To study RML in animals, exhaled breath condensate was collected using a special device (patent RU 134772 U1) and its volume generated in 1 minute and from 100 L of exhaled air was measured. It was found that in healthy calves, RML is 0.030-0.124 mL per minute and 0.242-1.506 mL per 100 L of exhaled air, in sick calves they increase on average by 24.3% (P <0.01) and 50.4% (P <0.001), respectively. Thus, airway inflammation increases RML in animals. Current results show that RML intensity in calves increases markedly with increasing body temperature, WI clinical score and correlates with pulmonary ventilation.

1. Introduction
Respiratory moisture loss (RML) in animals is associated with the release of water vapour filtered from the vessels of the small circle of blood circulation and upper respiratory tract, and liquid secretions formed in the alveoli and bronchi [1, 2]. The exhaled aerosol is formed by condensation of supersaturated water vapour (volatile compounds that are soluble in water are removed with it) and dispersion of non-volatile substances of the bronchoalveolar fluid [1, 2].

One way to study respiratory moisture loss (RML) in animals is to collect exhaled breath condensate (EBC) and measure the volume of EBC generated per unit time from a known volume of exhaled air [1]. During the EBC collection procedure, the animal breathes in a special mask equipped with a system of valves that do not allow the inhaled and exhaled air to mix [1]. The exhaled air passes through a condenser, where it is cooled by melting ice [2] or a refrigerator device [3], and is collected in a storage container.

The growing interest in studying EBC, supported by numerous publications on this topic [1, 2], is explained not only by the known advantages of this RML research method (accessibility, non-invasiveness, and others) but also by its potential diagnostic capabilities. Airway inflammation and acid-base balance disorders accompanying bovine respiratory disease (BRD) [4, 5] can alter RML, increasing or decreasing their intensity in animals [1]. At the same time, the collection of EBC in calves has not yet been unified. The method of RML research using EBC is only being formed [1, 3].
This work aimed to study the indicators that characterize RML in neonatal calves, and their relationship with the clinical signs of BRD.

2. Methods

The object of the study was 85 Holstein calves aged 2-28 days (weighing from 35 to 60 kg): 66 healthy and 19 with clinical signs of BRD. The animals were evaluated using the clinical scoring system WI developed by veterinarians at the University of Wisconsin at Madison [6]: the rectal temperature was measured, and the presence and nature of induced and (or) spontaneous coughing, nasal discharge, eye discharge, head and ear condition were assessed. Also, the respiratory rate and heart rate per minute were measured in all animals. Calves with a clinical score of 3 points or less were considered healthy [6].

To study RML in calves, we used a device for collecting EBC in animals, patent RU 134772 U1 (shown in figure 1).

![Figure 1. Procedure for collecting EBC from a calf.](image)

The breathing mask allows you to hermetically fix the device on the face of the calf, and the inhalation and exhalation valves in its composition do not allow the inhaled and exhaled air to mix during spontaneous breathing and direct the flow of exhaled air through a silicone tube to a removable storage container made of polypropylene and placed in a refrigerator. The cooling chamber and silicone tubes connecting the storage container with the exhalation valve of the respiratory mask and the spirometer are enclosed in heat-insulating casings made of foamed polyethylene with a closed cellular structure, which insulates them from the environment, positively affecting the rate of condensation of exhaled air vapours, and allows for a more complete collection of EBC. The SSP spirometer (KPO Medaparatura, Ukraine) as part of the device allows you to measure respiratory minute volume (RMV) and the tidal volume directly during the EBC collection procedure, which is necessary for further calculation of RML indicators. The EBC collection procedure for each calf took 20 minutes. The temperature (14°C) and humidity (60%) of the inhaled air in the experiment were kept constant for all animals. The calf exhaled air passed through the exhalation valve into the front chamber of the respiratory mask and then passed through the tube to a removable storage container, immersed in a cooling chamber with melting ice, where it cooled and condensed, turning into EBC. From the storage
container, the exhaled airflow successively passed through the SSP tube and spirometer and entered the environment. The volume of EBC generated in 20 min ($V_{EBC}$) was measured immediately after the collection procedure. The average volume of EBC formed in a calf in 1 min ($V_{1EBC}$) and from 100 L of exhaled air ($V_{2EBC}$) was calculated based on the measured data according to the following formulas:

$$V_{1EBC} = \frac{V_{EBC}}{20}$$

$$V_{2EBC} = V_{1EBC} \cdot \frac{100}{RMV}$$

Statistical data processing was performed in the IBM SPSS Statistics 20.0 program (IBM Corp., USA). All data were expressed as an average ± standard deviation (median). The relationship between the studied parameters was determined using the non-parametric Spearman criterion. The significance of differences between groups of calves was determined using the Independent-samples Mann-Whitney U-test.

3. Results and discussion

The results are presented in table 1.

| Parameter | WI clinical score ≤ 3 | WI clinical score > 3 |
|-----------|-----------------------|-----------------------|
| $V_{EBC}$ (mL) | 1.503 ± 0.381 (1.480) | 1.842 ± 0.394 (1.840)$^b$ |
| $V_{1EBC}$ (mL) | 0.075 ± 0.019 (0.074) | 0.092 ± 0.020 (0.092)$^b$ |
| $V_{2EBC}$ (mL) | 0.744 ± 0.262 (0.702) | 1.227 ± 0.515 (1.056)$^c$ |

The data is presented as an average ± standard deviation (median).

$^b$ Differences between groups of calves are statistically significant at $P < 0.01$.

$^c$ Differences between groups of calves are statistically significant at $P < 0.001$.

It was found that healthy neonatal calves with calm, even breathing lose from 0.030 to 0.124 mL of moisture per minute, which is 43.2-178.6 mL per day. In terms of 100 L of exhaled air, their RML ranges from 0.242 to 1.506 mL. It was previously reported that $V_{2EBC}$ in healthy calves 24 hours after birth is 0.55 ± 0.09 mL [7] and does not change significantly during the first 14 days of life [2], in animals with a bodyweight of 50 to 150 kg - 1.4 ± 0.4 mL using an ECoScreen® condensing system (Jaeger, Hoechberg, Germany) to collect EBC [3]. In our experience, the parameters that characterize RML did not change statistically significantly in healthy animals from the 2nd to the 28th day after birth. From the literature, it is known that the parameter $V_{1EBC}$ in animals can increase with increasing EBC collection time [1, 3], as well as the gradient between the exhaled air temperature and the condenser temperature used to collect EBC (can vary from 0 to -20°C in different types of devices) [1]. Parameter $V_{1EBC}$ decreases with increasing air velocity [1, 3]. The nature of pulmonary ventilation, in turn, depends on the ambient temperature: an increase in temperature leads to an increase in the respiratory rate per minute and dead space ventilation compared to tidal ventilation [1, 3]. Taking these factors into account, in this work we have tried to standardize the EBC collection procedure as much as possible, maintaining a constant temperature (14°C) and humidity (60%) of the inhaled air, as well as the EBC collection time (20 minutes) in calves.

From the data presented in table 2, it can be seen that $V_{1EBC}$ and $V_{2EBC}$ in animals increased with increasing body temperature and did not depend on heart rate per minute. In calves with elevated body temperature ($\geq 39.5°C$), RML per minute was 23.3% higher ($P < 0.05$) compared with individuals with normal body temperature. $V_{1EBC}$ increased with an increase in pulmonary ventilation. At the same time, the parameter $V_{2EBC}$ showed an inverse dependence on the respiration rate per minute ($r = -0.291$, $P < 0.01$), RMV ($r = -0.737$, $P < 0.001$) and tidal volume ($r = -0.527$, $P < 0.001$). Our data are consistent with earlier studies that revealed in calves an inverse relationship between the parameters $V_{2EBC}$ and tidal volume [7], as well as a direct dependence of $V_{1EBC}$ on RMV [3].
In calves with BRD symptoms (WI clinical score > 3), the values of $V_{1EBC}$ and $V_{2EBC}$ were higher on average by 24.3% (P < 0.01) and 50.4% (P < 0.001), respectively, compared with healthy individuals. A direct relationship was found between the WI clinical score and $V_{1EBC}$ ($r = +0.260$, P < 0.05) and $V_{2EBC}$ ($r = +0.529$, P < 0.001) in calves.

**Table 2.** Correlation coefficients between the parameters characterizing RML and some clinical and physiological parameters in neonatal calves.

| Parameter                          | $V_{1EBC}$ | $V_{2EBC}$ |
|------------------------------------|------------|------------|
| Rectal temperature                 | +0.252$^a$| +0.218$^a$|
| Heart rate per minute              | -0.155     | -0.153     |
| The respiratory rate per minute    | +0.096     | -0.291$^b$|
| Respiratory minute volume          | +0.363$^b$| -0.737$^c$|
| Tidal volume                       | +0.259 a   | -0.527 c   |
| WI clinical score                  | +0.260 a   | -0.529 c   |

$^a$ P < 0.05.  
$^b$ P < 0.01.  
$^c$ P < 0.001.

The data presented in table 3 illustrate changes in RML parameters depending on the presence of induced or spontaneous cough in calves. In animals with induced and spontaneous cough, parameter $V_{1EBC}$ was higher by 31.1% (P < 0.01) and 24.3% (P < 0.01), and parameter $V_{2EBC}$ by 46.7% (P < 0.001) and 47.0% (P < 0.001), respectively, compared with healthy animals without coughing. Current results support the suggestion that airway inflammation increases RML [8]. Future research should focus on standardizing the method of collecting EBC in calves.

**Table 3.** Parameters characterizing respiratory moisture loss in calves, depending on the presence of cough.

| Parameter                        | $V_{1EBC}$ (mL) | $V_{2EBC}$ (mL) |
|----------------------------------|-----------------|-----------------|
| There is no cough (n = 66)       | 0.075 ± 0.019 (0.074) | 0.753 ± 0.269 (0.702) |
| Induced cough (n = 10)           | 0.097 ± 0.020 (0.097)$^b$ | 1.030 ± 0.193 (1.030)$^c$ |
| Spontaneous cough (n = 9)        | 0.087 ± 0.020 (0.092)$^b$ | 1.463 ± 0.717 (1.032)$^c$ |

The data is presented as an average ± standard deviation (median).

$^b$ Differences compared with the group of calves without coughing are statistically significant at P < 0.01.

$^c$ Differences compared with the group of calves without coughing are statistically significant at P < 0.001.

4. Conclusion
Thus, the intensity of RML in neonatal calves depends on body temperature and volume of pulmonary ventilation. In healthy animals aged 2-28 days, RML is 0.030-0.124 mL per minute and 0.242-1.506 mL per 100 L of exhaled air. Airway inflammation in calves is accompanied by an increase in RML of 24.3% and 50.4%, respectively. Moreover, the intensity of RML in them increases in direct proportion to the severity of BRD according to the WI clinical score. These findings open up new prospects for the development of non-invasive methods for the diagnosis and control of BRD in calves on a farm.
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