Early detection of bovine respiratory disease in calves by induced cough

A E Chernitskiy¹ and V A Safonov²,³

¹All-Russian Veterinary Research Institute of Pathology, Pharmacology, and Therapy, 114b, Lomonosova str., 114b, Voronezh, 394087, Russian Federation
²Voronezh State Agrarian University named after Emperor Peter the Great, 1, Mitchurina str., Voronezh, 394087, Russian Federation
³E-mail: safonovbio@gmail.com

Abstract. 100 Holstein calves aged 7-14 days investigated the effectiveness of various cough provocation methods for bovine respiratory disease early detection. The animals were evaluated according to the clinical scoring system WI, to provoke cough in calves, they performed: 15-minute run, 30-second apnea on exhalation, palpated the trachea in its lower third region and intravenously injected 0.6% hydrogen peroxide solution at 0.9 % sodium chloride solution at a dose of 0.4 ml per kg of body weight to induce H₂O₂-induced bronchoconstriction. Out of 100 calves, 60 were selected without spontaneous cough and with a WI clinical score of 3 points or less, in which the leukocyte count, differential leukocyte count, neutrophil-lymphocyte ratio, nuclear shift index, and pH of exhaled breath condensate and the hydrogen peroxide concentration in exhaled breath. For 2 weeks, these animals were monitored daily for the bovine respiratory disease signs. It was established that 30-second apnea and H₂O₂-induced bronchoconstriction allow diagnosing bronchitis in calves 6-12 days before its symptom complex (peak) development when other symptoms are still absent and WI clinical score is 3 points or less. However, already at this disease stage, animals exhibit leukocytosis, increased serum content of haptoglobin, decreased pH of exhaled breath condensate, and increased the hydrogen peroxide concentration in exhaled breath. The trachea lower third cough on palpation appearance indicates the tracheobronchitis early manifestations presence in calves. Trachea lower third Sequential palpation and 30-second apnoea to provoke coughing in calves allows not only the bovine respiratory disease early detection, but also to differentiate early bronchitis and tracheobronchitis manifestations.

1. Introduction
Bovine respiratory disease (BRD) is an important problem in dairy farming worldwide [1, 2]. The BRD is recorded up to 52% of all calves before weaning [3]. The economic damage from the BRD consists of the costs associated with treatment, culling and calves death, a decrease in their growth intensity, fertility and milk production in the future [4, 5]. The BRD development in calves is associated with adverse environmental factors combination, viral and bacterial pathogens, and stressors that suppress the host normal immune response [6, 7]. The BRD complex nature makes the creation of a universal gold standard for BRD diagnosis problematic [8, 9].

The BRD on-farm diagnosis, as a rule, is based on the disease subjective visual signs in a calf (depression, coughing, dyspnoea, nasal and ocular discharge) and measurement of rectal temperature
[10, 11], less often thoracic auscultation and ultrasonography [2, 9]. It is shown [11] that among all the respiratory tract damage symptoms in calves, the cough has the highest sensitivity (72.8%) and specificity (90.9%) for the BRD detecting. The BRD other symptoms in calves (tachypnea, dyspnea, nasal discharge, ocular discharge, fever) are less sensitive and specific [11]. The duration, soreness, frequency, cough manifestation strength and nature (dry or wet, dull or sonorous) make it possible to judge the expiratory muscles' strength, lung tissue elasticity, pathological process localization in the respiratory organs and the disease course nature [12, 13]. Depending on the duration, it is customary to distinguish between acute, subacute and chronic coughs, from excitation - productive, accompanied by sputum discharge, and unproductive (dry) [2, 12]. In the spontaneous cough absence in calves for the BRD diagnosis, it is induced artificially [2, 10]. To provoke a cough, you can use animals run for 15 minutes [2, 14], the trachea palpation [15, 16], 30-second apnoea [14, 17], irritating substances aerosols (monochloride iodine, triiodine-ethylene glycol, aluminium iodide and others) [2] or H$_2$O$_2$-induced bronchoconstriction [2, 18]. Probably provoking cough underestimating other methods, veterinarians and farmers, as a rule, use only tracheal palpation for the BRD early detection in calves [19, 20].

This work aim was to conduct a provoking cough various methods' effectiveness comparative assessment (15-minute run, 30-second apnoea, the trachea palpation and H$_2$O$_2$-induced bronchoconstriction) for the BRD early detection in calves.

2. Methods

100 Holstein calves aged 7-14 days were assessed according to the clinical scoring system WI, developed by veterinarians at the Wisconsin University at Madison [10]: the rectal temperature was measured, cough, nasal discharge, ocular discharge, head and ear position were assessed. Cough in animals was provoked in the following ways: they were run for 15 minutes [2, 14], a 30-second expiratory apnoea was performed (shown in figure 1) [14, 17], the trachea was palpated in the lower third region [16]. Then, venous blood and exhaled breath condensate (EBC) samples were obtained from the calves for laboratory testing.

![Figure 1. Conducting a 30-second expiratory apnoea to the calf.](image)

Blood samples from animals were taken by the jugular vein puncture in sterile vacuum tubes with EDTA and without anticoagulant, EBC was collected using a special device [21] for 20 minutes;
taking into account the EBC volume formed in 1 minute and from 100 L of exhaled breath [22]. After obtaining blood samples and EBCs to induce coughing, the calves were injected intravenously with 0.6% hydrogen peroxide solution in 0.9% sodium chloride solution at a dose of 0.4 ml per kg of body weight to induce H2O2-induced bronchoconstriction [18]. Based on the study results, 60 animals were selected out of 100 without spontaneous cough and with a WI clinical score of 3 points or fewer, of which 3 groups were formed with n = 20 each. In calves Group I, coughs could not be induced by the four methods any we used to provoke it. In individuals Group II, the trachea lower third palpation did not cause cough. However, the other three methods - a 15-minute run, a 30-second apnea and H2O2-induced bronchoconstriction - provoked a cough. In animals Group III, the cough was observed in all four methods of its provocation used in the experiment. The calves were monitored daily for 2 weeks: their condition was assessed on the WI scale [19], respiratory and heart rate using an SSP spirometer (KPO Medaparatura, Ukraine) and a mask with a valve system, respiratory minute volume was determined and tidal volume; took into account the symptoms timing, nature, severity and the BRD duration.

Laboratory tests were performed on venous blood and EBC samples from 60 calves remaining in the experiment. After clotting for 1 h at room temperature, blood samples without anticoagulant were centrifuged (UC-1612, ULAB, China) at 4000 × g for 10 min at room temperature [23] and serum were carefully harvested and stored at −20 °C until biochemical analysis. The leukocytes total number, differential leukocyte count, the haptoglobin concentration in the serum were investigated in the animals’ blood; the pH level (HI1230B, HANNA, Germany) and the hydrogen peroxide concentration were measured in the EBC. To determine the differential leukocyte count, a venous blood drop was thinly spread over a glass slide, air-dried, and stained with Romanowsky stain by the May – Grunewald – Giemsa technique. Then two hundred blood cells were counted, classified, and their percentage was determined [24]. The neutrophil-to-lymphocyte ratio (NLR) and the nuclear shift index (NSI) were calculated as the ratio of the sum of myelocytes, metamyelocytes, and stab neutrophils to segmented neutrophils [25]. Serum haptoglobin was quantified on a UV-1700 spectrophotometer (Shimadzu, Japan) by the formation of a haptoglobin-haemoglobin complex with the addition of horse haemoglobin (Sigma-Aldrich, USA) using the rivanol method [26]. The hydrogen peroxide concentration in EBC in calves was determined on an RF-5301 PC spectrofluorometer (Shimadzu, Japan) at an irradiation wavelength of 568 nm and an emission wavelength of 581 nm, using the Amplex Red Ultra fluorescent dye (Invitrogen, USA) [27], then the peroxide content was calculated hydrogen in 100 L exhaled breath (EB) [27].

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. The calves difference between groups significance was determined using the Independent-samples Mann-Whitney U-test.

3. Results and discussion

The calves blood and EBC laboratory tests results are presented in table 1.

From table 1, it can be seen that at the experiment beginning in the calves of Group I, haematological and biochemical signs of inflammation were absent. The content of leukocytes in the blood, NSI, NLS and serum content of haptoglobin in them corresponded to the age norm [26, 28]. Throughout the observation period, body temperature, respiratory rate, heart rate, respiratory minute volume, and tidal volume were within physiological limits [14, 29], and the clinical assessment on the WI scale did not exceed 3 points. Its lower third region trachea palpation, running for 15 minutes and 30 seconds of apnoea on expiration, as well as H2O2-induced bronchoconstriction, did not cause cough.

From the data in table 1, it can be seen that the animals of Group II had leukocytosis with the leukocyte formula shift to the left, a decrease in the EBC pH, a haptoglobin increased content in the blood serum [26] and hydrogen peroxide in the exhaled breath [30, 31]. All the above indicated the airway inflammation presence in calves [2]. After 6–12 days, a bronchitis symptom complex (peak) development was observed. Clinically, this manifested itself as mixed dyspnoea, spontaneous cough
with sputum production, dry or moist large and fine bubbling rales during the chest and serous nasal discharge auscultation, rectal temperature was increased (up to 40 °C).

Table 1. The blood and EBC laboratory parameters in calves at the experiment beginning.

| Parameter               | Group I (n = 20) | Group I (n = 20) | Group I (n = 20) |
|-------------------------|------------------|------------------|------------------|
| Leukocytes \(10^9/L\)  | 6.29±0.61        | 9.48±2.50\(^a\) | 10.98±2.45\(^a\) |
| NSI                     | 0.21±0.08        | 0.36±0.06\(^a\) | 0.36±0.06\(^a\) |
| NLR                     | 0.41±0.19        | 0.57±0.08        | 0.59±0.15        |
| Haptoglobin (g/L)       | 2.42±0.92        | 4.27±0.76\(^a\) | 4.76±0.97\(^a\) |
| pH EBC                  | 7.54±0.11        | 6.59±0.18\(^a\) | 6.38±0.13\(^a\) |
| \(\text{H}_2\text{O}_2\) (nmol/100 L EBC\(^b\)) | 0.25±0.08        | 0.72±0.12\(^a\) | 0.78±0.14\(^a\) |

The data is presented as an average ± standard deviation.

\(^a\) Differences are statistically significant compared to Group I at \(P < 0.05\).

The blood and EBC laboratory studies results in calves of Group III (presented in table 1) indicated an inflammatory process' presence in the respiratory organs [2, 31]. After 8.2 ± 0.9 days, they observed a tracheobronchitis symptom complex development: a sonorous painful cough, dry or moist large-bubble rales in the trachea and bronchi, serous-catarrhal nasal discharge, the trachea hypersensitivity during palpation along its entire length, tachypnoe (up to 48 breaths per minute) and tachycardia (up to 100 heartbeats per minute).

Our study showed that the trachea palpation and calves clinical assessment on the WI scale is not always the best tools for the BRD early detection. Thus, 30-second apnea on exhalation and intravenous administration of 0.6% hydrogen peroxide solution on 0.9% sodium chloride solution at a dose of 0.4 ml per kg of body weight in the experiment allowed calves to diagnose bronchitis early manifestations, 6-12 days before its symptom complex (peak) development, when other signs of the disease were still absent, and according to the clinical assessment on the WI scale (0-3 points), the animals could be considered healthy from the respiratory system [10, 19]. The mechanism \(\text{H}_2\text{O}_2\)-induced bronchoconstriction is based on the reactive oxygen species physiological effects in the respiratory system [18]. It was experimentally established [32, 33] that at physiological concentrations the superoxide anion does not cause changes in the trachea and bronchi diameter, while hydrogen peroxide and hydroxyl radicals cause contraction or relaxation, respectively, depending on the concentration. At concentrations exceeding physiological, superoxide anion causes the trachea and bronchi relaxation, while hydrogen peroxide and hydroxyl radicals cause predominant contraction [32, 34]. The latter, with the trachea and bronchi mucous membrane inflammation, due to their increased sensitivity, leads to a cough [18].

4. Conclusion

Thus, a 30-second expiratory apnoea and \(\text{H}_2\text{O}_2\)-induced bronchoconstriction make it possible to diagnose bronchitis in calves 6-12 days before its symptom complex (peak) development, when other signs of the disease are still absent, but, according to the clinical assessment on the WI scale (0-3 points), the animals are considered healthy. The cough appearance the trachea lower third on palpation indicates the tracheobronchitis early manifestations presence in calves, and its middle and upper third on palpation — tracheobronchitis and tracheitis late stages. The trachea lowers third Sequential palpation and 30-second apnoea on exhalation to provoke coughing in calves allows not only the BRD early detection, but also to differentiate bronchitis and tracheobronchitis early manifestations.
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