Evaluation of the measurement of leukotriene B$_4$ concentrations in exhaled condensate as a noninvasive method for assessing mediators of inflammation in the lungs of calves

Petra Reinhold, Dr med vet, PhD; Gunther Becher, Dr med; Michael Rothe, Dr rer nat

**Objective**—To determine whether measurement of an inflammatory mediator in exhaled condensate could provide a noninvasive method for evaluating lungs of calves.

**Animals**—84 calves ≤ 2 months old.

**Procedure**—Concentration of leukotriene B$_4$ (LTB$_4$) was evaluated in the exhaled condensate of healthy calves and calves with experimentally induced respiratory tract infections. For collection of samples of exhaled condensate, the total amount of exhaled air was directed into a cooled double-jacketed tube. Each tube was sealed and stored at –80°C. The LTB$_4$ concentration was measured, using an ELISA.

**Results**—In exhaled condensates of clinically healthy calves, normally distributed and highly reproducible LTB$_4$ concentrations (mean ± SD, 116.1 ± 55.4 pg/ml) were measured. After experimentally induced infection with *Pasteurella multocida* serovar D, LTB$_4$ in exhaled condensate increased significantly (mean, 179% increase), compared with basal concentrations before infection; this increase in LTB$_4$ was significantly correlated with deterioration in lung function. In 2 of 4 calves experimentally infected with bovine respiratory syncytial virus, the LTB$_4$ concentration in exhaled condensate increased (300 to 400% increase), compared with baseline values, which was associated with development of bronchial hyperresponsiveness after infection.

**Conclusions and Clinical Relevance**—Collection of exhaled condensate is tolerated well by calves and is an acceptable method for obtaining fluid from exhaled air originating from the lungs. This method provides alternatives for diagnosing and evaluating treatment of naturally acquired and experimentally induced diseases of the lungs and airways in calves. (Am J Vet Res 2000;61:742–749)

Invasive techniques (ie, bronchoalveolar or tracheobronchial lavage, lung or bronchial biopsy) are usually required to provide samples for analysis of immunologic or inflammatory mediators released by the lungs or airways. Collecting samples of condensate of exhaled air (ie, exhaled condensate) offers a noninvasive alternative, which has been described in humans.$^{1-3}$ This method does not affect the typical breathing pattern of subjects. Therefore, it could be applied to conscious and spontaneously breathing animals. Although exhaled condensate does not contain cells, a wide range of inflammatory mediators or markers of cellular activity (eg, leukotrienes, cytokines, or markers of oxidant stress) have been detected in exhaled condensate of humans.$^{1-3}$ To the best of our knowledge, collection of samples of exhaled condensate in spontaneously breathing domestic animals has not been described. Thus, the purpose of the study reported here was to validate this noninvasive technique in healthy and pneumonic calves. Attention was focused primarily on only 1 substance (leukotriene B$_4$ [LTB$_4$]), which is a non-specific mediator of inflammation. Objectives of the study were to adapt the technique of sample collection of exhaled condensate for use in cattle, to examine the LTB$_4$ concentration in exhaled condensate samples of clinically healthy calves, to evaluate the reproducibility of LTB$_4$ concentrations in exhaled condensate, to compare LTB$_4$ concentrations in exhaled condensate and bronchoalveolar fluid, and to study changes in LTB$_4$ concentrations in calves with experimentally induced bacterial and viral respiratory tract infections.

**Materials and Methods**

**Animals**—All calves used in the study reported here (n = 84) were ≤ 2 months old. Calves were maintained in accordance with standard practices used to reduce transport stress. Nutritional supplements, hay, and water were supplied. Daily clinical observation (including monitoring of respiratory rate, nasal secretions, ocular secretions [tears], rectal temperature, appetite, and body weight) was used to confirm the clinical status of calves before they were included in the study. All experiments were performed in conscious calves; none of the calves was anesthetized or sedated.

**Procedure for collection of samples of exhaled condensate**—The system for collection of samples described by Becher et al was adapted for use in spontaneously breathing animals. A special face mask with 2 valves was designed so that the calves were allowed to inspire ambient air through the inspiratory valve. Exhaled air was directed by a nonrebreathing valve into a cooled (−15 to −20°C) double-jacketed sample collection tube (length, 55 to 60 cm; inner diameter, 10.0 mm) made of polytetrafluoroethylene. Duration of sample collection for each calf was ≥ 30 minutes. Immediately after collecting a single sample of exhaled condensate, the tube was sealed at both ends and stored immediately at −80°C until analyzed.
Measurement of LTB4—Before measuring LTB4 concentrations, samples were thawed, and the mass of each sample of exhaled condensate was recorded. Leukotriene B4 concentration was measured by use of a commercially available ELISA\(^a\) that has been described as being highly specific for LTB4. Cross-reactivity for other leukotrienes or other products of arachidonic acid metabolism (eg, prostaglandins, thromboxanes) was < 0.1%. Range of measurement for the ELISA was 6 to 800 pg/ml. Results for the assay were reproducible (r = 0.76; R² = 0.58) for 2 repeated LTB4 measurements.

Protocol for correlation of exhaled condensate with the pattern of breathing—Samples of exhaled condensate (n = 43) were collected at constant ambient conditions (ambient temperature, 18°C; ambient relative humidity, 60 to 69%; respiratory rate, 30 to 40 breaths/min; tidal volume, 10 to 15 ml/kg; respiratory rate, 7 breaths/min). Each sample was collected on 2 consecutive days in 13 calves (17 to 33 days old; body weight, 40 to 56 kg). As an expression of variability, the coefficient of variation for 2 consecutive days was calculated for each calf. This type of variability includes within-calf variability and reproducibility. In addition, the responsiveness of the bronchial system of each calf was evaluated after collection of the second sample of exhaled condensate. Therefore, a stepwise bronchial challenge test (inhalation of aerosolized carbachol solution that involved use of 3 increasing dosages) was performed.

Long-term variability of LTB4 concentration in exhaled condensate and comparison with concentration in bronchoalveolar lavage fluid—Three clinically healthy 4-week-old calves that weighed (mean ± SD) 54.3 ± 12.5 kg to enable us to study the physiologic range of LTB4 concentrations. To avoid collecting samples of exhaled condensate from calves with respiratory tract disease, each calf was evaluated for normal calf reproducibility and variability of LTB4 concentrations.

Concentrations of LTB4 were measured in exhaled condensate of clinically healthy calves—Samples of exhaled condensate were collected from 35 clinically healthy calves (from 29 to 50 kg). During collection of exhaled condensate, rectal temperature and several variables of ventilatory function (tidal volume, minute volume, respiratory rate) were recorded for each calf. Regression analysis was used to clarify whether the quantity of exhaled condensate obtained was correlated with variables of ventilatory function or rectal temperature.

Measurement of LTB4 concentration in exhaled condensate of clinically healthy calves—Samples of exhaled condensate were collected from 35 clinically healthy calves that weighed (mean ± SD) 54.3 ± 12.5 kg to enable us to study the physiologic range of LTB4 concentrations. To avoid collecting samples of exhaled condensate from calves with respiratory tract disease, each calf was evaluated for normal calf reproducibility and variability of LTB4 concentrations.

Effect of viral infection on respiratory tract function—Four clinically healthy calves (23 to 25 days old, weighing 40 to 56 kg) were inoculated with bovine respiratory syncytial virus (BRSV 375, ATCC No. VR-1339), using an aerosol procedure, as described elsewhere.\(^b\) Before inoculation and up to 2 weeks after inoculation, each calf was examined clinically by evaluation of rectal temperature, respiratory rate, appetite, cough, and ocular and nasal discharges twice daily. During the same period, a daily lung function test, using the IOS, was performed on each calf. For LTB4 measurement, exhaled condensate was collected before inoculation as well as 1, 2, and 3 days and 1 and 2 weeks after inoculation in all calves. After each collection of exhaled condensate, bronchial reactivity was tested in each calf, using a stepwise bronchial challenge test (inhalation of aerosolized carbachol solution that involved 5 increasing dosages).

Non-specific bronchial reactivity test—To characterize bronchial responsiveness, a non-specific bronchial challenge test was performed in each calf in 2 of the aforementioned groups. Each test involved inhalation of aerosolized carbachol solutions of 5 increasing dosages as follows: inhalation of 10 L of aerosolized 0.1% carbachol solution, inhalation of 15 L of aerosolized 0.1% carbachol solution, inhalation of 5 L of aerosolized 0.5% carbachol solution, inhalation of 10 L of aerosolized 0.5% carbachol solution, and inhalation of 15 L of aerosolized 0.5% carbachol solution. The carbachol solutions were aerosolized by means of a jet nebulizer and reservoir (plastic bag with a maximum volume of 10 L). Calves inspired the calculated aerosol volume from this reservoir via the inspiratory valve of the face mask. More than 60% of the particles in the inhaled aerosol were < 3 μm in diameter.

After each increment in the challenge test, response to carbachol was determined conventionally by use of an esophageal balloon catheter for measurement of intrapleural pressure and a Lilly-type pneumotachograph for measurement of airflow. Total pulmonary resistance and dynamic lung compliance were calculated in accordance with the method of Mead and Whitehafter.\(^c\) Bronchial challenge tests were stopped when intrapleural pressure increased to > 150% of baseline values. After cessation, total pulmonary resistance increased, and dynamic lung compliance decreased, reflecting a considerable bronchoconstrictive effect induced by carbachol.

Statistical analysis—For statistical analysis, the Student t-test for paired observations and an ANOVA (least-significant difference) were used. Coefficients of linear correlation...
and equations of linear regression were identified, using the linear model of regression analysis. Rank correlations among variables were analyzed by use of Spearman rank correlation coefficients, a procedure that uses the ranks of the data rather than the actual data.

**Results**

Dependency of exhaled condensate on the pattern of breathing—Because of differences in body size and clinical status, calves had differing patterns of breathing and differing rectal temperatures. For example, respiratory rate ranged from a minimum of 17 breaths/min to a maximum of 53 breaths/min. Tidal volume varied between 223 and 493 ml, and minute volume varied between 4.3 and 15.0 L. Rectal temperature ranged between 38.3 and 40.6 °C for these calves. The quantity of exhaled condensate obtained within 30 minutes for each calf varied between 0.1 and 2.8 ml.

Significant associations were not detected between the quantity of the exhaled condensate and rectal temperature, using analysis of rank correlation or linear correlation. In contrast, significant linear correlations existed between the quantity of exhaled condensate and variables of ventilatory function as well as between the quantity of exhaled condensate and body weight, as determined by use of linear regression analysis (Table 1). Despite an interaction between body weight and ventilatory volumes, results clearly indicated that the quantity of exhaled condensate depended mainly on the respired volume per time (ie, minute volume).

Concentration of LTB₄ in exhaled condensate of clinically healthy calves—Concentrations of LTB₄ in exhaled condensate were normally distributed. The LTB₄ concentrations in samples of exhaled condensate obtained from 35 clinically healthy calves ranged from 31.8 to 225.4 pg/ml (mean ± SD, 116.1 ± 55.4 pg/ml).

Short-term variability of LTB₄ concentration in exhaled condensate—To study the within-calf variability of LTB₄ concentrations in exhaled condensate, 13 calves were examined on 2 consecutive days. Of these 13 calves, 1 had a considerably higher sensitivity to inhalation of carbachol (ie, bronchial hyperresponsiveness), compared with that of the other 12 calves; data for that calf were excluded for statistical analysis. Whereas, mean LTB₄ concentrations in exhaled condensate on days 1 and 2 were 109 and 123 pg/ml, respectively, in the 12 healthy calves with typical bronchial sensitivity, the hyperresponsive calf had LTB₄ concentrations > 400 pg/ml in exhaled condensate on each day. Within the group of 12 calves, differences were not detected between results of day 1 or 2 (eg, neither the obtained volumes of exhaled condensate nor LTB₄ concentrations differed significantly between sample collection days). The within-calf coefficient of variability of LTB₄ concentrations in exhaled condensate in samples obtained on 2 consecutive days varied between 2.9 and 25.9% (mean, 11.6%). A significant reproducibility (ie, linear correlation) was found between LTB₄ concentrations of day 1 and 2 within the group of 12 healthy calves (Fig 1).

Long-term variability of LTB₄ concentration in exhaled condensate and BALF—Within each clinical healthy calf, the LTB₄ concentration was highly reproducible, and there was low variability in concentrations in exhaled condensate and BALF samples during the 7 to 8 weeks of the study. However, the absolute concentration of LTB₄ in exhaled condensate had a pattern that differed from that in BALF in specific calves. Although the LTB₄ concentrations were similar in exhaled condensate and BALF in 1 calf, the concentration was higher in BALF than in exhaled condensate in 2 calves (Fig 2).

Total number of cells, alveolar macrophages, polymorphonuclear neutrophils, and lymphocytes were determined for all BALF samples. Values for 10 BALF samples from 3 calves were included in the study.
Samples were collected from 2 calves on days 1, 21, and 43 and from 1 calf on days 4, 18, 32, and 53. Total number of cells (range, 380,000 to 490,000 cells) and number of alveolar macrophages (range, 376,000 to 483,000 alveolar macrophages) were highest on the first day of collection in 2 calves and highest on the second day of collection in 1 calf. All calves had the lowest total number of cells (range, 142,000 to 245,000 cells) and the lowest number of alveolar macrophages (range, 141,000 to 223,000 alveolar macrophages) on the last day of sample collection. Number of neutrophils was highest on the first day of collection and lowest on the last day of collection in 2 calves; however, number of neutrophils was lowest for the second day of collection and highest for the last day of collection in the other calf. Number of lymphocytes was lowest on the first day of collection for all 3 calves; however, the highest number of lymphocytes was detected on the last day of collection for 1 calf but not for the other 2 calves.

A strong linear correlation was found between the number of neutrophils and the LTB₄ concentrations in BALF ($r = 0.93; P < 0.001; \text{Fig 3}$). In contrast, significant correlations did not exist between LTB₄ concentrations and total number of cells or number of alveolar macrophages.

Experimentally induced bacterial infection—All calves developed pneumonia after inoculation with $P. \text{multocida}$ serovar D. Five days after inoculation, visible lesions for these 7 calves ranged from 4 to 32% (median, 13%) of the total lung surface per calf. Clinically, the respiratory rate increased (25 ± 3 breaths/min before inoculation, 44 ± 16 breaths/min after inoculation), and tidal volume significantly ($P = 0.03$) decreased (648 ± 93 ml before inoculation, 460 ± 179 ml after inoculation). Changes in respiratory mechanics were characterized by a significant

### Table 1—Equations of linear regression and correlation coefficients ($r$ and $R^2$) between quantity of exhaled condensate obtained during 30 minutes, variables of ventilatory function, body weight, and rectal temperature in 43 calves

<table>
<thead>
<tr>
<th>Equation of linear regression</th>
<th>$r$</th>
<th>$R^2$ (%)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{co}$ (ml/30 min) = −0.271 + 0.0484 $RR$ (breaths/min) $^*$</td>
<td>0.41</td>
<td>16.66</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>$E_{co}$ (ml/30 min) = −0.255 + 0.0031 $V_t$ (ml) $^*$</td>
<td>0.33</td>
<td>10.71</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>$E_{co}$ (ml/30 min) = −0.918 + 0.2103 $V_{min}$ (L) $^*$</td>
<td>0.63</td>
<td>40.11</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>$E_{co}$ (ml/30 min) = 1.570 + 0.0603 body weight (kg)</td>
<td>0.45</td>
<td>20.09</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>$V_t$ (ml) = 117.8 + 6.09 body weight (kg)</td>
<td>0.44</td>
<td>18.99</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>$V_{min}$ (L) = 0.804 + 0.19 body weight (kg)</td>
<td>0.51</td>
<td>25.90</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*$^*$Interaction with body weight.

$E_{co}$ = Amount of exhaled condensate. $RR$ = Respiratory rate. $V_t$ = Tidal volume. $V_{min}$ = Minute volume.

### Figure 3—Correlation between LTB₄ concentration and number of neutrophils in 10 samples of BALF obtained periodically from 3 clinically normal calves (calf A = ○, calf B = ◇, calf C = ●) during a period of 7 to 8 weeks. Values were significantly ($r = 0.93; R^2 = 85.6%; P < 0.001$) correlated, using the following equation of linear regression: $LTB_4$ (pg/ml of BALF) = 71.64 + 0.0042 neutrophils/ml of BALF. —— = Line of linear regression. ——— = 95% Confidence limits for the regression line. ———— = 95% Prediction limits for new observations.

### Figure 4—Respiratory impedance expressed as mean ± SD resistance (R) and mean ± SD reactance (X) before and after experimentally induced infection with $P. \text{multocida}$ serovar D in 7 calves. *Values differed significantly ($P < 0.01$) from values before infection. $kPa/(L/s)$ = Pressure/flow relationship as unit for R and X.
(P = 0.01) increase in resistance and a decrease in reactance (Fig 4). Compared with basal concentrations before inoculation (59.6 ± 17.2 pg/ml), LTβ4 concentrations in exhaled condensate significantly (P = 0.03) increased after bacterial infection (95.7 ± 22.1 pg/ml), a mean increase of 179%.

Reactance values between 5 and 25 Hz and LTβ4 concentrations in exhaled condensate were significantly negatively correlated (rSpearman = −0.56 to −0.68; Table 3). Resistance values, respiratory rate, and tidal volume were not significantly correlated with LTβ4 concentrations in exhaled condensate.

Experimentally induced viral infection—Clinical signs of respiratory tract infection (ocular and nasal discharge, mild cough) were observed in each calf after BRSV inoculation. Rectal temperature and nasal discharge, mild cough) were observed in each calf after BRSV inoculation. Rectal temperature increased significantly (P < 0.01). Changes in lung function were characterized by a slight but significant increase in respiratory rate, a significant decrease in tidal volume, and a significant decrease in lung compliance (Table 3). Results of the carbachol challenge test, which was performed to classify the bronchial reactivity before and after infection, were determined. Before infection, all 4 calves were able to tolerate the carbachol challenge up to 10 or 15 L of aerosolized 0.5% carbachol solution. By 1 week after BRSV infection, carbachol responsiveness to carbachol inhalation did not change in the other 2 calves, when compared with baseline reactivity.

Concentrations of LTβ4 in samples of exhaled condensate before BRSV infection ranged from 68.3 to 124.9 pg/ml. After infection, LTβ4 concentration increased by approximately 300 to 400%, compared with baseline values in the 2 calves with postinfectious bronchial hyperreactivity (Fig 3). However, in the 2 calves that did not develop bronchial hyperreactivity, LTβ4 concentrations did not increase substantially after BRSV infection.

**Table 3**—Changes in variables of respiratory function, rectal temperature, and body weight in 4 calves inoculated with bovine respiratory syncytial virus (BRSV)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before inoculation</th>
<th>7 days after inoculation</th>
<th>14 days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory rate (breaths/min)</td>
<td>17.3 ± 3.8*</td>
<td>28.0 ± 7.3*</td>
<td>21.3 ± 3.6*</td>
</tr>
<tr>
<td>Tidal volume (ml)</td>
<td>656 ± 136*</td>
<td>462 ± 125*</td>
<td>652 ± 110*</td>
</tr>
<tr>
<td>Tidal volume/body weight (ml/kg)</td>
<td>12.6 ± 1.6*</td>
<td>8.5 ± 1.5</td>
<td>10.0 ± 0.5</td>
</tr>
<tr>
<td>Lung compliance (L/kPa)</td>
<td>1.78 ± 0.26*</td>
<td>1.63 ± 0.16</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td>Lung compliance/body weight (L/kPa/kg)</td>
<td>0.035 ± 0.005*</td>
<td>0.029 ± 0.003*</td>
<td>0.031 ± 0.004*</td>
</tr>
<tr>
<td>Rectal temperature (C)</td>
<td>38.8 ± 0.05*</td>
<td>39.7 ± 0.10</td>
<td>39.4 ± 0.17</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>51.9 ± 7.8*</td>
<td>56.5 ± 7.6</td>
<td>65.9 ± 7.9*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD.

* *Within a row, values with different superscript letters differ significantly (P < 0.05; ANOVA).

* *Within a row, values with different superscript letters differ significantly (P < 0.01; ANOVA).
in the airways could provide a diagnostic method for evaluating the respiratory tract noninvasively. In the study reported here, the measurement of LTB4 concentrations in the condensate of exhaled air was validated in healthy and pneumonic calves.

The general principle of collecting samples of exhaled condensate in spontaneously breathing domestic animals is comparable to that used in humans. The only difference exists in the way the collection system is adapted for animals. Because animals breathe predominantly through the nose, substances originating from the nasal mucosa may be included in the condensate.

To clarify the theoretical possibility of contamination of exhaled condensate with saliva, Becher et al compared the activity of amylase and fluid viscosity in samples of exhaled condensate, BALF, and saliva from human volunteers. Measurement of viscosity and amylase activity documented that saliva was not included in exhaled condensate, even when exhaled condensate was collected during forced expiration (ie, 30 consecutive forced vital capacity maneuvers). In animals, the probability of contaminating exhaled condensate with saliva is less than that in human beings because of nasal breathing. Nevertheless, the face mask needed for use in spontaneously breathing animals should be designed to allow retention of saliva.

As documented in the study reported here, the volume of exhaled condensate obtained depended directly on the breathing volume over time. In contrast, breathing volume and amount of condensate were not correlated in humans. This difference could be caused by a smaller range of values for ventilatory variable in adult humans, compared to that for calves of differing body weights that had a range for minute volume of 4 to 15 L. On the basis of results for our study, sample collection duration can be determined for each animal, taking the minute volume of breathing and the cooling temperature of the equipment into consideration.

Differing materials and qualities of the sample collection tubes have been compared. For measurement of LTB4 concentrations, recovery rate was excellent with polytetrafluoroethylene, less with polyethylene, and least with borosilicate glass. Thus, the sample collection procedure should be standardized with regard to the surface of the sample collection tube.

Comparing exhaled condensate and BALF in humans with lung diseases, interleukin (IL)-1β was found in comparable concentrations in BALF and exhaled condensate, whereas IL-6 had a 5-fold higher concentration in BALF compared with exhaled condensate. However, to our knowledge, a direct comparison has not been published concerning leukotrienes in BALF samples and breath condensate. As documented here, LTB4 measurements in exhaled condensate and BALF were highly reproducible within each calf over a period of ≥ 7 weeks. In BALF samples, LTB4 concentration correlated with the number of neutrophils in the lungs but not with the number of macrophages. This observation suggests that the LTB4 detected may primarily be produced by neutrophils in the lungs, at least in healthy calves. However, there is evidence from in vitro studies that bovine alveolar macrophages are involved in arachidonic acid metabolism and also produce LTB4.

Two of the 3 calves in our study had increased LTB4 concentrations in BALF compared with concentrations in exhaled condensate. Because exhaled air does not contain cells, only the products of cellular metabolism are expected in exhaled condensate. In contrast, BALF contains a large number of cells capable of releasing and destroying leukotrienes. Consequently, various concentrations of mediators could be expected in BALF when these samples are not stored or analyzed immediately after collection. Therefore, differences in processing of samples may explain the absolute difference between LTB4 concentrations in exhaled condensate and BALF in the study reported here. Furthermore, exhaled condensate should always be collected prior to collection of BALF in the same subject, because collection of BALF may modify the environment within the lungs. It has been reported that lavage procedures lead to an influx of neutrophils in the lungs as well as changes in lung surfactant for up to 3 weeks after bronchoalveolar lavage.

In healthy humans, LTB4 concentrations have been measured in serum, plasma, arterial blood, and BALF. In exhaled condensate of healthy subjects, LTB4 concentrations < 200 to 300 pg/ml have been reported. In accordance with those findings, the study reported here revealed that low but considerable concentrations of LTB4 (< 200 to 250 pg/ml) also were found in exhaled condensates and BALF of healthy calves. It can be assumed from reports that LTB4 may play a physiologic role in the endocrine regulation of the bronchial system or regulation of the immune system of the lungs. However, production of LTB4 in clinically healthy humans and other animals also may reflect permanent activity of bronchial defenses, probably stimulated by inhaled pathogenic organisms, chemical pollutants, or irritative substances. Using exhaled condensate, it can be expected that inflammatory mediators of respiratory epithelium would be detected earlier during the course of disease in ill animals and animals with subclinical disease, compared to techniques that measure functional or structural changes.

Because of its chemotactic activity for neutrophils (but also for eosinophils, monocytes, and lymphocytes), LTB4 has been identified as a potent mediator of allergic and inflammatory diseases. In humans, leukotrienes have been related to asthma and chronic lung diseases (eg, chronic obstructive pulmonary disease, cystic fibrosis, sarcoidosis). To our knowledge, data have not been reported on exhaled LTB4 concentrations in animals with respiratory tract infections. As documented in the study reported here, bacterial and viral infections led to a significant increase in LTB4 concentrations in exhaled condensate in calves. However, the percentage increase was much higher for BRSV infection (300 to 400%), compared with the increase for P multocida infection (179%). Compared with LTB4 concentrations in healthy calves, the increased LTB4 concentrations after bacterial infection were still within the reference range for healthy calves. Thus, only within-calf comparisons before and after infection were able to document the increase in
LTB₄ concentrations attributable to infection. A study comparing 2 groups of subjects would have failed to reveal a difference and would have led to false-negative results. On the basis of these data, the involvement of LTB₄ in bacterial respiratory tract infections seems to be only moderate.

In the study reported here, a significant association was not detected between LTB₄ concentration and respiratory rate, tidal volume, or respiratory resistance. These findings were not unexpected. Although release of LTB₄ is believed to be a primary response to the stimulation of inflammatory cells, changes in the ventilatory pattern (respiratory rate, tidal volume) or in respiratory mechanics should occur as a result of a complex regulatory response that includes local release of bronchoconstrictive and dilatatory mediators, local reflex mechanisms in the airways, and neural responses. However, a significant negative linear correlation was found between the increase in LTB₄ concentrations and the decrease in reactance after induced bacterial infection. In contrast to respiratory resistance, which reflects predominantly resistive properties of central bronchi and extrathoracic airways in calves, reactance has been identified as a more sensitive variable that reflects particularly resistive properties of small airways and capacitative properties of the lungs. Therefore, the increase in LTB₄ concentration in the exhaled condensate was associated mostly with changes in respiratory mechanics located in the peripheral part of the respiratory system in the calves of our study. This result is in agreement with findings of other investigators who observed an increase in trapping of pulmonary gas in guinea pigs after experimental LTB₄ inhalation. According to the findings in that study, LTB₄ can induce delayed onset of airway obstruction that is stereospecific, cyclooxygenase-independent, and temporally associated with influx of granulocytes into lung airways.

Although respiratory syncytial virus causes mild or severe respiratory disease in human infants and young children, the closely related BRSV causes a similar disease pattern in calves. In both children and calves, pathophysiologic changes during respiratory syncytial virus-induced bronchiolitis result in inflammatory obstruction of small airways leading to changes in respiratory mechanics. Bronchioli may collapse or be filled with exudate. Consequently, parts of the lungs can be atelectatic or hyperinflated. In all calves experimentally inoculated with BRSV in our study, comparable changes in clinical findings and similar changes in respiratory function (decrease in lung compliance) was detected after inoculation. Despite these similarities, profound differences were observed in airway responsiveness. A relevant increase in bronchial responsiveness was observed in only 2 of 4 calves after BRSV inoculation. This is in accordance with reports for humans indicating that bronchiolitis attributable to respiratory syncytial virus may result in bronchial hyperreactivity, but this condition does not develop in all patients. Despite a plethora of controlled studies, mechanisms for this virus-induced bronchial hyperreactivity have not been completely identified. Because LTB₄ is not a marker of contractile activity of the airways, airway inflammation seems to be the link between LTB₄ and bronchial hyperreactivity. Evans et al documented an effect of LTB₄ on neutrophil influx and activation in the airways after allergen challenges in asthmatic inflammation. Recently, IL-8 was found to cause bronchial hyperresponsiveness and neutrophil accumulation in airways of guinea pigs, and these effects were caused by the release of LTB₄. In addition, LTB₄ is a potent neutrophil chemoattractant that also stimulates eosinophils in vitro, and eosinophils are involved in airway hyperresponsiveness and in conditions associated with respiratory syncytial virus infection. Consequently, there seems to be evidence for an association between LTB₄ concentrations and the degree of nonspecific airway responsiveness accompanied by airway inflammation or an influx of inflammatory cells in the airways.

References
8. Nowak D, Antonycz A, Kröl M, et al. Increased content of

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