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Original article

The effect of respiratory diseases on serum lactate dehydrogenase and its isoenzyme patterns in calves

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Abstract

In this study we examined the serum activity of lactate dehydrogenase (LDH) and its isoenzyme patterns in 28 calves of a lowland black spotted breed and its crossbreeds at the age of 2-6 months suffering from clinically noticeable manifested respiratory diseases – bronchopneumonia (BRD Group). As a control group we used 35 clinically healthy calves of the same age, breed and nutrition (Healthy Group). The sick calves did not show clinical signs or pathological lesions on other organ systems. The results found in sick calves showed a significantly higher total activity of LDH than in clinically healthy animals ($P < 0.01$). The mean activity of LDH was 2012 U/l in healthy calves and in calves with respiratory diseases 2529 U/l. The differences in all LDH isoenzyme patterns between both groups of animals were significant ($P < 0.001$) and in calves with respiratory diseases are characterized by a marked increase of the LDH 1 fraction and a decrease in the proportion of the other four LDH isoenzymes. Our results differ from those observed and presented in respiratory diseases in human medicine or in sheep. The explanation for the obtained results in calves and the determination of their diagnostic significance needs further studies and investigations using more animals with various severity of clinical signs and pathological changes, including analysis and determination of lactate dehydrogenase isoenzyme patterns in healthy and affected cattle lung tissue.

Key words: calves, respiratory diseases, lactate dehydrogenase, isoenzymes, electrophoresis

Introduction

Lactate dehydrogenase (LDH) is an enzyme which catalyses the interconversion of lactic and pyruvic acids using the NAD^+ coenzyme. It is widely distributed in the body, high activities are found in the heart, liver, skeletal muscle, kidney, and erythrocytes;

lesser amounts are found in the lung, smooth muscle, and brain. Because of its widespread activity in numerous body tissues, LDH is elevated in a variety of disorders (Johnson-Davis and McMillin 2010). There are many conditions which contribute to increased activity, and an elevated total LDH value is a rather non-specific finding. Therefore, LDH assays assume

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more clinical significance when separated into isoenzyme fractions. In human medicine the LDH isoenzymes are mostly used in the diagnosis of pathological conditions in cardiology, hepatology, haematology and oncology (Huijgen et al. 1997). LDH isoenzyme profiles were the first isoenzyme profiles used in clinical veterinary medicine in an attempt to detect specific organ damage (Kramer 1989). Even though LDH and its isoenzyme examinations are not routinely used in veterinary laboratory diagnosis, many reports have suggested their usefulness in animals. Many literature sources and reports dealing with the analysis of LDH from the aspect of the physiology of animals and pathophysiology of metabolic and systemic disorders indicate that the activity of LDH and its isoenzyme patterns show a great variation between animal species and also tissue distribution (Beatty and Doxey 1983, Preuss et al. 1989, Yasuda et al. 1990, Heinova and Blahovec 1994, Heinova et al. 1996). Therefore, it is necessary to understand the composition and distribution of the tissue isoenzymes of each animal. In addition to commonly used blood serum or plasma, LDH activities were also analysed in other body fluids of animals, e.g. synovial fluid (Schmökkel et al. 2001), cerebrospinal fluid (Nečas and Sedláková 1999), milk (Babaei et al. 2007), and bronchoalveolar lavage fluid (Davies et al. 1986, Weiss et al. 1991, Reinhold et al. 1992). In cattle there are many reports suggesting their use in experimental investigations, as well as in the diagnosis of organ and metabolic diseases (Dawra et al. 1991, Sobiech and Kuleta 2002, Enemark et al. 2004, Lubojacka et al. 2005, Tanaka et al. 2006, Bellova et al. 2009). However, in contrast to the high incidence of respiratory diseases in animals, and mainly in cattle, only a very limited number of reports describe the diagnostic value of such measurement in veterinary medicine. On the other hand, in human medicine there are many reports and literature sources regarding the usefulness of LDH and its isoenzyme patterns as indicative of lung damage or inflammation in various respiratory diseases (Drent et al. 1996).

The aim of the present study was to determine the serum activity of lactate dehydrogenase and its isoenzyme patterns in calves suffering from clinically manifested diseases of the respiratory tract and evaluate the possible effect of the diseases on the extent of changes comparing the obtained results with values found in healthy animals.

Materials and Methods

Animals

The study included 28 calves of a lowland black spotted breed and its crossbreeds at the age of 2-6 months and body weight from 65 to 158 kg suffering

from respiratory tract diseases of various severity. The sick calves came from the university school farm or were submitted to the Clinic for ruminants of the University of veterinary medicine and pharmacy in Košice by a local veterinarians from dairy farms. We included in the evaluation calves with noticeable clinical manifestation of signs of bronchopneumonia. The diagnosis was done using standard physical clinical examination procedures and in some cases by ultrasound and endoscopic examination by the same veterinarian. Clinical examination was oriented predominantly to assessment of the general health (body temperature, feed intake, behaviour, body condition) and the respiratory system by visual inspection (breathing rate, nasal discharges, type of breathing, signs of dyspnoea, dry or wet spontaneous coughing) and auscultation (increased or decreased loudness of the breathing sounds, bronchial sounds, additional abnormal breathing sounds – crackles, wheezes, and signs of laboured breathing with open mouth). The calves did not show clinical signs or pathological lesions on other organ systems. At the clinic we performed further therapy of sick calves. For the purpose of comparison of the analysed variables between sick (BRD Group) and healthy calves, we also included in the investigation 35 clinically healthy calves of the same age, breed and nutrition (Healthy Group).

Collection of blood samples

Blood samples for the investigation were taken from animals by direct puncture of *v. jugularis*. The blood was collected from sick animals once during the study after the clinical examination and including calves with obvious clinical signs of bronchopneumonia in the study. Blood was collected into disposable blood collection plastic tubes with gel activator. Blood samples were allowed to clot at room temperature, and the serum was recovered after centrifugation at 3000 g for 30 minutes. The harvested serum was stored at room temperature and analysed within 24 hours.

Assays of serum analysis

Activity of total lactate dehydrogenase (LDH; EC 1.1.1.27) was determined in blood serum using commercial diagnostic kits (Randox) and an automatic biochemical analyzer ALIZE (Lisabio, France). In this analysis, pyruvate as a substrate is reduced to lactate at a pH of 7.5 and temperature of 37°C. The LDH isoenzymes were separated by agarose gel electrophoresis on a buffered agarose gel at pH 8.4

Table 1. Total LDH activity (U/l) and isoenzyme patterns of LDH (%) in evaluated groups of calves.

Group of calves	LDH	LDH isoenzymes					
		LDH 1	LDH 2	LDH 3	LDH 4	LDH 5	
Healthy	x	2012	41.1	30.5	17.4	6.8	4.3
	SD	424	2.8	1.5	1.3	0.9	0.8
	median	2006	41.1	30.6	17.3	6.7	4.5
	min.	1114	34.7	27.3	15.0	4.5	2.6
	max.	2838	46.0	32.6	20.4	8.6	6.1
BRD	x	2529	53.7	28.1	12.3	3.6	2.2
	SD	758	3.3	2.3	1.6	0.7	0.6
	median	2464	53.9	27.7	12.7	3.6	2.2
	min.	1246	45.9	23.2	9.2	2.2	1.3
	max.	4940	59.9	34.7	15.9	5.1	3.8
P <	0.01	0.001	0.001	0.001	0.001	0.001	

BRD – calves with respiratory diseases; P – significance of the differences of means between the evaluated groups.

according to the total activity of LDH. The serum samples for the analysis of isoenzyme patterns were diluted at a ratio 1:4 or 1:5 with 0.9% saline solution. The electrophoresis was performed using an automated agarose gel electrophoresis system Hydrasys (Sebia Corporate, Evry-Paris, France) using commercial diagnostic kits Hydragel 7 ISO-LDH (Sebia Corporate, Evry-Paris, France) according to the procedure described by the manufacturer. Ten microliters of each serum sample were applied to numbered sample wells on the agarose gel. Control serum (Enzycontrol, Sebia Corporate, Evry-Paris, France) was included in each run of samples. The electrophoretic migration was performed for 8 minutes at 20°C constantly at 10 W, 36 mA, and 280 V. After migration, lactate was used as a specific substrate to detect enzyme activity. LDH isoenzymes were visualized by nitroblue tetrazolium reduction to formazan, where the amount of resulting formazan precipitate is proportional to the LDH enzymatic activity. The electrophoretic gels were scanned, and the LDH isoenzymes were visualized and displayed on densitometry system Epson Perfection V700 (Epson America Inc., Long Beach, California, USA) by light transmission and automatic conversion into an optical density curve presentation. The different LDH isoenzymes were identified according to their position (LDH1 the fastest fraction, and LDH5 the most cathodic), and quantified by computer software Phoresis version 5.50 (Sebia Corporate, Evry-Paris, France) and, if necessary, corrected by visual inspection of the electrophoretogram. The relative concentrations of the individual LDH isoenzymes were determined as a percentage of the optical absorbance.

Statistical analysis

Statistical evaluation of the results was performed by assessment of the average values (x), standard deviations (SD), medians, and the range of the results (minimum and maximum values) in the monitored groups of calves. The significance of the differences in the means (P) of corresponding variables between the groups was evaluated using an unpaired Student's test. Statistical analysis was done by using the program GraphPad Prism 5 vers. 5.04. (GraphPad Software Inc.).

Results

The means of total LDH activity and the percentage of each LDH isoenzyme, as well as medians and the ranges of the individual values in clinically healthy

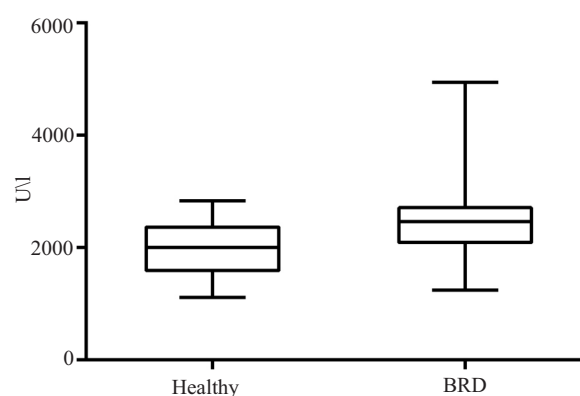


Fig. 1. Total serum activity of LDH in evaluated groups of calves (BRD – calves with respiratory diseases). The plots show the median (line within box), 25th and 75th percentiles (box), minimum and maximum values (whiskers).

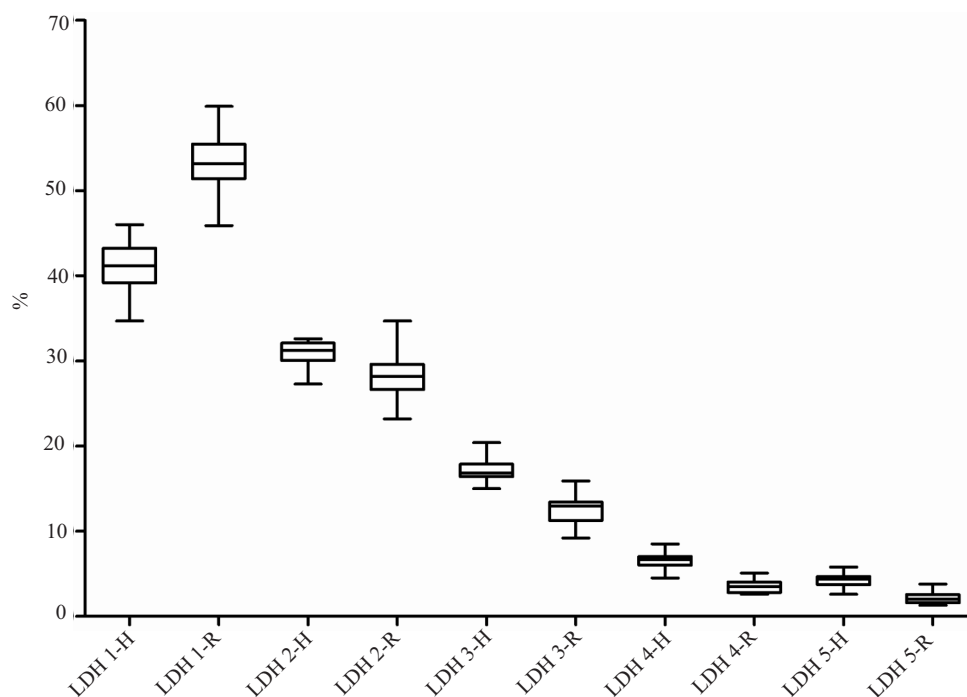


Fig. 2. Isoenzyme of LDH in evaluated groups of calves (H-healthy calves, R-calves with respiratory diseases). The plots show the median (line within box), 25th and 75th percentiles (box), minimum and maximum values (whiskers).

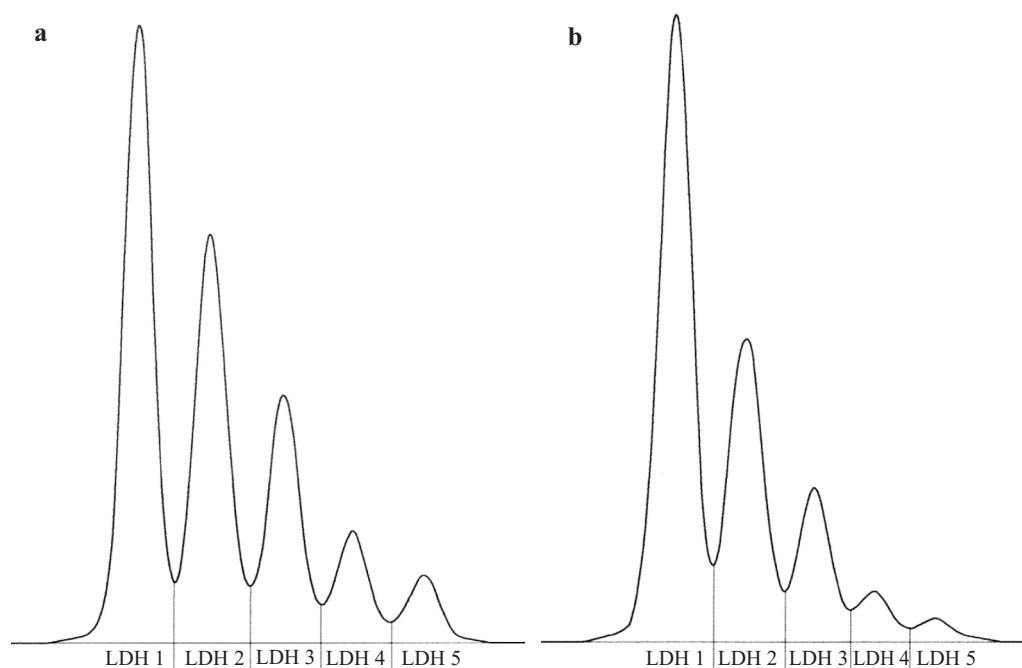


Fig. 3. Electrophoretic patterns of LDH isoenzymes in serum of healthy calf (a) and calf suffering from respiratory disease (b)

calves and calves suffering from respiratory diseases are presented in Table 1. Analysis of the distribution of the individual values of the total LDH activity and its isoenzyme patterns are shown in Fig. 1 and 2. Differences in the electrophoretic patterns of LDH isoenzymes in healthy and sick animals are shown in Fig. 3.

The mean total activity of LDH in calves with clinical signs of respiratory diseases was significantly higher than in clinically healthy ones ($P < 0.01$, Table 1). The individual values of total activity of LDH in the group of healthy animals ranged from 1114 to 2838 U/l, and in the group of sick animals from 1246 to 4940 U/l. Analysis of the individual results of samples showed

that 50% of values in healthy calves were in the range of 1593 to 2365 U/l, whereas in sick animals the results ranged from 2090 to 2712 U/l (Fig. 1). The distribution of the LDH isoenzymes between the evaluated groups of calves showed statistically high significant differences in all five LDH isoenzymes ($P < 0.001$, Table 1). The most marked change in the proportion of LDH isoenzymes was found in LDH 1. The mean percentage of LDH 1 isoenzyme in calves suffering from respiratory diseases was considerably higher, whereas the mean proportion of the other four LDH isoenzymes was lower (Fig. 2). While the individual values of the LDH 1 isoenzyme ranged from 34.7 to 46.0% in healthy calves, in animals suffering from respiratory diseases the range of results is from 45.9 to 59.9%. Further analysis of the individual results of the isoenzyme LDH 1 showed that 50% of values in healthy calves were in the range from 38.8 to 43.6%, whereas in sick animals the results ranged from 51.6 to 55.7%. Among the other four LDH isoenzymes, LDH 3, LDH 4 and LDH 5 showed more remarkable lowering when compared to isoenzyme LDH 2. This typical noticeable shift of the isoenzymatic activity towards the LDH 1 fraction and differences in the other four isoenzymes in sick calves are shown in Fig. 3.

Discussion

Changes in plasma or serum enzymes and isoenzymes are useful indicators of tissue damage in many diseases. Enzyme increases are usually related to their leakage from damaged cells. The amount of increase depends on such factors as the concentration of enzyme in the cells, the rate of leakage of enzyme from cells, and the rate of plasma enzyme clearance (Boyd 1983). Cellular enzymes in the extracellular space are still of benefit because they serve as indicators suggesting disturbances of the cellular integrity induced by pathological conditions. The use of biomarkers in medicine lies in their ability to detect disease and support diagnostic and therapeutic decisions. They also have a potential value as an important prognostic tool. Clinically useful biomarkers can supplement the clinical diagnosis and help monitoring of the disease, evaluation of treatments and prediction of prognosis and health outcome. In humans the activity and release of a large body of inflammatory mediators and markers of cell damage such as lactate dehydrogenase have been evaluated as prognostic and monitoring tools of the disease development, activity and progression also in respiratory diseases (Tzouveleakis et al. 2005).

Respiratory diseases in cattle are common and are serious health problems which affect mostly the lower

respiratory tract, the lung. However, in animals there are limited blood biochemistry variables useful for the laboratory analysis of the degree and extent of lung damage. According to present knowledge, mainly from human medicine, LDH is one of these potential parameters presented in the literature as a possible indicator of lung damage (Drent et al. 1996). Lactate dehydrogenase is a cytoplasmatic enzyme present in all major organs. Serum LDH is abnormal in many disorders due to its widespread distribution in the body. Each organ or tissue contains LDH isoenzymes in a characteristic proportion. High concentrations of LDH are found in the liver, cardiac and skeletal muscle, erythrocytes, gut, and renal cortices. Although assay of total serum LDH activity is by no means tissue specific, appearance of specific LDH is frequently diagnosed in liver or heart damage (Clarenburg 1992). The fact that this enzyme is widely distributed in the body raises doubts about its relevance in medicine and identifying clinical situations in which the determination of LDH and its isoenzymes in serum are of real value (Huijgen et al 1996).

Knowledge of the serum (plasma) activity and isoenzyme patterns of LDH in clinically healthy animals is a prerequisite for the evaluation of pathological conditions. In the present study in healthy calves the highest proportion was found in isoenzyme LDH 1 and was followed in decreasing order by isoenzymes LDH 2, LDH 3, LDH 4, and LDH 5. These results correspond with the findings presented in cattle by Kim and Cho (1989) and Sobiech et al. (2002). The highest proportion of isoenzymes was obtained for LDH 1 and LDH 2 and the lowest for LDH 4 and LDH 5. Our results slightly differ in the LDH 4 and LDH 5 isoenzyme proportion in inverse order to that presented by Sobiech and Kuleta (2006). The highest activity, by a slight margin, was demonstrated for LDH 2 isoenzyme by Salplachta and Necas (2000).

For the diagnosis of systemic diseases in ruminants, the analysis of LDH activity and its isoenzyme patterns in blood serum were mostly used in animals with liver and muscle damage (Sobiech and Kuleta 2002, Lubojacka et al. 2005), changes in isoenzyme patterns were found in diarrhoeic calves by Sobiech and Kuleta (2006), and elevation of plasma LDH activity without any changes in the isoenzyme patterns was observed by Dawra et al. (1991) in animals with enzootic bovine haematuria. Determination of LDH activity and its isoenzyme proportion was also used as a means of pregnancy detection and for examination of periparturient changes in dairy cows (Peter et al. 1987).

However, there are scarce sources in the litera-

ture regarding the activity of LDH and its isoenzymes in ruminants and mainly refers to cattle suffering from respiratory diseases. In sheep inoculated with parainfluenza virus type 3 and *P. haemolytica* Davies et al. (1986) found in bronchoalveolar lavage (BAL) fluid increased levels of LDH, probably reflecting increased cell damage. Weiss et al. (1991) detected in lavage fluid increases of LDH activities in calves with experimentally induced pneumonic pasteurellosis. Increased total activity of LDH in lavage fluid in calves obtained from pneumonic lesions was recorded also by Reinhold et al. (1992) and the levels of LDH activity positively correlated with the severity of the lung damage.

As blood samples are more commonly used in animals for laboratory analyses, the purpose of the present study was therefore to investigate the possible usefulness of these enzymes in blood serum as an indicator for lung damage. Lung tissue, like other organ tissues, is rich in LDH and the tissue levels are about 500 fold higher than those normally found in serum (Drent et al. 1996). This should be the assumption for its release from damaged cells and elevation of blood activity of LDH and changes in proportion of isoenzyme patterns. Studies of homogenates from human lungs demonstrated that LDH 3 and LDH 4 are the most widely distributed LDH isoenzymes in this tissue (Galen and Gambino 1975). In the normal equine lung LDH 3 and LDH 4 have been found to constitute approximately equal proportions of the LDH activity with lower proportions of the other three isoenzymes (Thornton and Lohni 1979). Milne and Doxey (1987) found, in canine lung tissue, the lowest proportion of LDH isoenzyme for LDH 1 and the highest for LDH 3. There are only a few reports regarding LDH isoenzyme distribution in ruminants, and these mainly refer to cattle lung tissue. While Beatty and Doxey (1983) found in lambs the highest LDH isoenzyme fraction for LDH 3 and LDH 1, and the lowest proportion was obtained for LDH 2, Milne and Doxey (1984) reported the lowest percentage in normal areas of lamb lungs for LDH 5 isoenzyme. They measured the total LDH and the percentage level of its isoenzymes also in lung lesions from lambs with acute and chronic pneumonia and found higher total enzyme activity mainly with increases of activity of LDH 4 and LDH 5, particularly in chronic pneumonia. They consider lung lesion as a potential for altering the serum isoenzyme distribution. Kim and Cho (1989) determined the activity in various cattle tissues and found the highest activity in the lungs. In normal calf lung tissues Salplachta and Necas (2000) demonstrated the highest LDH isoenzyme proportion for LDH 2, LDH 1 and LDH 3.

In human medicine, elevations of serum LDH 3 occur most frequently with pulmonary involvement and are also observed in patients with various carcinomas (Johnson-Davis and McMillin, 2010). Elevation of LDH 3 isoenzyme was found also by Hagadorn et al. (1971) in rats after experimentally induced immunologic lung injury. These authors suggest that LDH 3 isoenzymes are released into the circulation from the cells in the damaged lung. In horses suffering from respiratory diseases Sommer et al. (1986) investigated the serum activity of enzymes and found increased activity of LDH. Georgiev and Monov (1976) reported an increase in total serum LDH and a considerable rise mainly in the activity of the isoenzymes LDH 1 and LDH 2, which they considered to be characteristic for pigs affected with bronchopneumonia.

In the present investigation, in calves with clinical signs of lower respiratory tract diseases we found significantly higher total activity of LDH than in clinically healthy animals, which corresponds to some of the above-mentioned findings. However, our results differ in the LDH isoenzyme patterns found in sick animals. In contrast to the results presented in humans or other species of animals they are characterized by a remarkable increase of the LDH 1 fraction and a significant decrease in the proportion of the other four LDH isoenzymes. We recorded a noticeable shift of the isoenzymatic activity towards the LDH 1 fraction. The increase in LDH activities indicated possible injury to the respiratory tract epithelium and release of LDH from epithelial cells lining the airways, which should be an indicator of damage to those cells. Inflammation of the lung is followed by influx of polymorphonuclear cells and activation of alveolar macrophages and also by changes in alveolar-capillary barrier permeability (Drent et al. 1996). The specificity of LDH isoenzyme patterns of these infiltrating cells could also be responsible for the species differences in the changes of LDH isoenzyme distribution. This assumption could be indicated by the results of LDH isoenzyme patterns in cow peripheral leukocytes presented by Tanaka et al. (2006).

In conclusion the presented results found in calves suffering from respiratory diseases are different from those presented in respiratory diseases in humans or sheep and shed further light onto the pathogenesis and pathophysiology of respiratory diseases in cattle. They indicate that this biomarker might be useful in laboratory diagnosis of bovine respiratory diseases. However, the explanation of the obtained results in calves and the determination of their diagnostic significance needs further studies and investigations in animals with various severity of clinical signs and pathological changes, including determination of lac-

tate dehydrogenase isoenzyme patterns in healthy and affected lung tissue or cells involved in the pathological inflammatory process.

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