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Research Article

Nutritional Indicators in Holstein Dairy Heifers Infected with Respiratory Syncytial Virus with Referring to Changes in Lipid Profile, Tumor Necrosis Factor-α and Acute Phase Proteins

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A B S T R A C T

In both experimental and natural calf pneumonia serum lecithin:cholesterol acyltransferase (LCAT) activity is reported to decrease, which may be due to involvement of cytokines in respiratory infection. This study aimed to evaluate whether a similar phenomenon occurs in dairy heifers naturally infected with Bovine Respiratory Syncytial Virus (BRSV) and in addition, to assess the relevance of LCAT to other metabolites, including Tumor Necrosis Factor α (TNF-α). An outbreak of BRSV infection happened in 12 dairy heifers on a farm and sera were obtained at days 0, 3, 7 (acute phase), 22 (convalescent phase) and

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50 (postconvalescent phase). Serum concentrations of haptoglobin (Hp) and α1-acid glycoprotein (AGP) were remarkably increased in the acute phase, which reflected the severity of the inflammatory process due to pneumonia. However, they gradually decreased after therapy and returned to normal from day 22. Reduced serum activities of LCAT and increased serum concentrations of TNF-α were also found at days 3 and 7, respectively, compared with the postconvalescent day (day 50). This reduced LCAT activity is considered to be related to the increase of serum TNF-α because TNF-α inhibits the synthesis of mRNA of LCAT in the liver. On the other hand, the significant elevation of the serum apolipoprotein A-I (apoA-I) concentration at day 0 compared with day 50 may suggest that there is also an increased serum Hp concentration because apoA-I has affinity for Hp. The change of serum LCAT activity found in this study is involved with the changes of TNF-α, apoA-I and Hp in inflammatory pathogenesis.

INTRODUCTION

Respiratory disorders are also a relevant health problem in young cattle, especially those kept indoors (Virtala *et al*., 1996; Nikunena *et al*., 2007), and they affect both the health and welfare status of the animal and the economic results of the producers. On the other hand, main clinical signs of respiratory disease in cattle include nasal discharge, coughing, fever, hampered respiration, inappetence, and depression. Signs may vary from subclinical to severe, which may lead to calf death. In addition to calf mortality and medical treatment costs, respiratory disorders imply economic losses due to slower growth of calves compared with healthy animals (Snowder *et al*., 2006).

Bovine Respiratory Syncytial Virus (BRSV) is a common cause of respiratory disease and a risk factor for the Bovine Respiratory Disease (BRD) complex (Jim, 2009). This virus is cytopathogenic and directly damages the respiratory epithelium in vivo, but also enhances host responses that play a critical role in the development of disease (Viuff *et al*., 2002).

Lecithin:cholesterol acyltransferase (LCAT) is mainly synthesized by the liver and in plasma, catalyzes the transesterification of Free Cholesterol (FC) with lecithin (phosphatidylcholine), resulting in Cholesteryl Ester (CE) (Jonas, 1998). The enzyme is activated by apolipoprotein A-I that is mainly distributed in the High-Density Lipoproteins (HDL). FC is taken up by HDL from the extrahepatic tissue surface as a substrate for LCAT. The produced CE is transferred to Low-Density Lipoprotein (LDL), and taken up by the liver or steriodogenic tissues, then converted to bile acids or steroid hormones (Albers *et al*., 1976).

LCAT activity is decreased in both experimental (Nakagawa and Katoh, 1999) and natural cases (Nakagawa and Katoh, 2001) of calf pneumonia, in which the expression of cytokines is assumed to be involved. In diseased calves, the serum CE concentration, particularly that in the HDL fraction, is concurrently decreased (Nakagawa and Katoh, 1999).

The reduced activity of LCAT during the inflammatory process is partially due to a decreased level of enzymes in plasma. A possible explanation for the reduction in LCAT activity is that hepatic synthesis and/or secretion of LCAT is inhibited by Tumor Necrosis Factor (TNF) or other cytokines. TNF has been shown to inhibit gene transcription and secretion of several hepatic proteins (Perlmutter *et al*., 1986).

Studies on experimental infections have shown that some Acute Phase Proteins (APPs) have good properties as markers of respiratory

infections in calves after viral (Heegaard *et al*., 2000; Grell *et al*., 2005), bacterial (Schroedl *et al*., 2001; Ganheim *et al*., 2003; Dowling *et al*., 2004) or combined challenge (Ganheim *et al*., 2003). However, only limited data are available on APPs as disease markers of spontaneous BRD. These studies have mainly been conducted to evaluate the potential of APPs as individual or herd health diagnostic tools in veterinary practice. Wittum *et al*., 1996; Young *et al*., 1996) reported that bovine APP haptoglobin (Hp) had limited capacity as a diagnostic clinical tool for BRD in feedlot cattle. Later, Hp was considered useful for identifying beef calves with BRD needing treatment and for monitoring treatment efficacy (Carter *et al*., 2002; Humblet *et al*., 2004), whereas serum amyloid A (apoSAA) and α1-acid glycoprotein (AGP) was not found to be a useful marker of BRD in feedlot calves (Carter *et al*., 2002; Berry *et al*., 2004).

The current study aimed to describe the clinical findings, haematological picture, serum biochemical changes including mainly LCAT activity, Hp and AGP and the most common isolated detected virus during the different stages of respiratory infections in naturally infected heifers starting from the onset of clinical signs before receiving any therapy till complete recovery of the animals. This study also aimed to evaluate whether decrease of serum LCAT activity occurs in dairy heifers naturally infected with BRS virus and, in addition, to assess relevance of LCAT to other metabolites including TNF-α and apolipoprotein (apoA-I), and other inflammatory biomarkers including APPs in these diseased dairy heifers.

MATERIALS AND METHODS

Animals: Twelve dairy heifers (6 to 7 months old) fed in Chitose, Hokkaido, Japan were used in this study. These animals were discovered with clinical signs of respiratory disorders by a farmer. These signs spread to all twelve heifers that were fed in the same pen within a few days. The other cattle were not fed in the same pen. These animals had not received any vaccination for infectious respiratory diseases. They were introduced to Rakuno Gakuen University, Veterinary Teaching Hospital for the initial medical examination. The diseased animals were examined and sampled at days 0, 3, 7, 22 and 50 from the outbreak. All cattle were treated under the Laboratory Animal Control Guidelines of Rakuno Gakuen University, which basically conform to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health in the USA (NIH publication No. 86-23, revised 1996).

The diseased heifers were classified into acute (day 0, 3 and 7), convalescent (day 22) and postconvalescent (day 50) phases. They were dealt with as follows: at day 0, no treatment; at days 3 and 7, the diseased heifers received antibiotics (benzylpenicillin and cephalothin sodium) and fluid therapy (Ringer's solution and 5% or 25% glucose solution); at day 22, only heifers with fever received antibiotics and fluid therapy; at day 50, all heifers had recovered after medical treatment.

Blood sampling: All blood samples were collected from the jugular vein into plain vacutainer tubes, and then were centrifuged at 3000 rpm for 15 min. Sera were separated and stored at -20ºC till analysis.

Methodology: Clinical examination of all heifers was conducted using clinical charts according to Rosenberger (1990). The serum concentration of Hp was determined using ELISA (Nakagawa *et al*., 1997). Single radial immunodiffusion assay was used to estimate the serum concentration of AGP (Itoh *et al*., 1990). The serum activity of LCAT was

measured by using a commercial kit (Dai-Ichi Pure Chemical, Tokyo, Japan). The serum concentration of bovine TNF-α was determined by sandwich ELISA using affinity-purified immunoglobulin Gs (IgGs) specific for the bovine cytokine (Hagiwara *et al*., 2000). Serum Total Cholesterol (TC) and FC levels were analyzed using commercial kits (Wako Pure Chemical, Osaka, Japan). The CE was estimated by subtracting the FC concentration from that of TC. Serum concentrations of nonesterified fatty acids (NEFA), phospholipids (PL) and triglycerides (TG) were measured using commercial kits (Wako Pure Chemical, Osaka, Japan). The serum apolipoprotein A-I (ApoA-I) concentration was determined by ELISA (Oikawa and Katoh, 1995).

Detection of pathogens: For presera (day 0) and postsera (day 22) from diseased heifers, the Virus Neutralization (VN) test was carried out using heat-inactivated sera in a microtiter system to determine antibodies titers to bovine herpesvirus 1 (BHV1) (van Drunen Little-van den Hurk *et al*., 1985), bovine viral diarrhea virus (BVDV) (Howard *et al*., 1987; Magar *et al*., 1988), Bovine Respiratory Syncytial Virus (BRSV) (Westenbrink *et al*., 1985), bovine adenovirus 7 (BAd7) (Mayr *et al*., 1970), Bovine Corona Virus (BCV) (Tsunemitsu *et al*., 1991) and bovine parainfluenza virus 3 (BPIV3) (Van Wyke Coelingh *et al*., 1988).

Statistical analysis: All statistical analyses were performed using SPSS version 17.0 (Chicago, USA). The data obtained from biochemical analyses were analyzed by repeated measures analysis of variance (ANOVA). The significance of differences between the means at selected sampling days and day 50 (postconvalescent) was evaluated by Dunnett's test. Values were expressed as means±SD.

RESULTS AND DISCUSSION

All affected heifers had antibodies for BRSV in day 0 and day 22. Eight of the twelve heifers had significant increases of the titer (more than 4-fold) in day 22. All heifers were negative (titer: less than 2) for IBR and BVDV. Six of the 12 heifers had serum antibodies for BPIV3, but they had no significant titer increase in day 22. Ten of the 12 heifers had serum antibodies for BAd-7, but only one heifer had a significant increase in the postserum antibody titer. Based on these results, the respiratory disease in heifers was diagnosed as BRSV infection. The percentage of the diseased heifers groups with seropositive reactors for RS virus among the other viruses was tested by VN test was about 66.67%. So, incidence of BRSV seroconversion after an outbreak of respiratory tract disease in our study was reported to be as high as 66.67%.

Clinical findings of the diseased heifers (Table 1 and 2) varied with sampling day. The reported findings were fever, tachycardia, cough, nasal discharge, abnormal lung sounds, reduced

Table 1: Clinical findings in heifers infected with respiratory syncytial virus.

	Days after outbreak				
Variables	0	3	7	22	50
Fever (≥39.4^{O}C)	12 (100.00)*	11 (91.70)	10 (83.30)	6 (50.00)	0 (0)
Tachycardia (≥90 b/m)	10 (83.30)	12 (100.00)	11 (91.70)	10 (83.30)	0 (0)
Cough	7 (58.30)	5 (41.70)	6 (50.00)	1 (8.30)	0 (0)
Nasal discharge	3 (25.00)	2 (16.70)	4 (33.30)	1 (8.30)	0 (0)
Abnormal lung sound	8 (66.70)	11 (91.70)	9 (75.00)	0 (0)	0 (0)
Ruminal hypomotility	1 (8.30)	5 (41.70)	7 (58.30)	0 (0)	0 (0)
Dehydration	2 (16.70)	3 (25.00)	2 (16.70)	0 (0)	0 (0)

*Number of heifers (%).

Table 2: Clinical findings in heifers infected with respiratory syncytial virus.

	Days after outbreak				
Variables	0	3	7	22	50
Fever	Feverage	Feverage	Feverage	Feverage (50%)	Absent
Tachycardia	Tachycardia	Tachycardia	Tachycardia	Tachycardia	Absent
Cough	Dry cough	Moist cough	Moist cough	Almost absent	Absent
Nasal discharge sticky	Serous or mucoid Almost absent	serous or mucoid Absent	Profuse and		
Lung sound	Harsh vesicular dry râles	Moist râles	Moist râles	Vesicular	Vesicular
Ruminal motility	Normal	Reduced	Atony	Normal	Normal
Dehydration	Observed	Observed	Observed	Absent	Absent

Table 3: Serum biochemical variables in heifers infected with respiratory syncytial virus.

	Days after outbreak				
Variables	0	3	7	22	50
LCAT (U)	701.32±127.33	630.86±97.88*	771.37±117.85	865.36±157.26	829.08±68.37
TNF- α (ng/l)	14380.00±7140.00	19680.00±8260.00	26190.00±6460.00*	14060.00±2020.00	13760.00±1540.00
ApoA-I (g/l)	2.15±0.24*	2.03±0.23	1.79±0.29	1.88±0.28	1.87±0.26
TC (mmol/l)	7.77±2.71	7.66±1.55	7.45±1.89	8.11±1.63	8.19±1.53
FC (mmol/l)	1.96±0.56	1.78±0.30	1.82±0.31	1.93±0.26	1.84±0.25
CE (mmol/l)	5.81±2.17	5.88±1.27	5.64±1.61	6.18±1.12	6.35±1.28
TG (mmol/l)	1.18±0.26	1.10±0.42	1.18±0.39	1.31±0.40	1.14±0.35
PL (g/l)	0.87±0.32	0.94±0.12	0.92±0.14	1.01±0.11	1.02±0.13
NEFA (mmol/l)	0.27±0.17	0.28±0.14	0.29±0.14	0.21±0.02	0.21±0.01

*significant ($p < 0.05$) when compared with the values at day 50. LCAT: lecithin:cholesterol acyltransferase; TNF-α: tumor necrosis factor-α; apoA-I: apolipoprotein A-I TC: Total Cholesterol; FC: Free Cholesterol; CE: Cholestryl Ester; TG: Triglycerides; PL: Phospholipids; NEFA: Non-Esterified Fatty Acids.

Table 4: Serum concentrations of haptoglobin (Hp) and α1-acid glycoprotein (AGP) in heifers infected with respiratory syncytial virus.

	Days after outbreak				
Variables	0	3	7	22	50
Hp (g/l)	1.42±0.83 (12/12)	0.72±0.59 (12/12)	0.56±0.40 (7/12)	Undetected (0/12)	Undetected (0/12)
AGP (mg/l)	573.58±235.76*	609.92±226.40*	496.92±156.23	337.08±90.27	365.50±197.34

*significant ($p < 0.05$) when compared with the values at day 50.

ruminal contractility and dehydration. The abnormal lung sound varied according to course of infection; Harsh vesicular or dry râles at day 0, moist rales at days 3 and 7 and Vesicular sound at days 22 and 50. The severity of clinical signs was aggravated from mild to severe degree during the course of infection until day 7. Thereafter, typically clinical signs disappeared gradually as the diseased heifers started to recover by day 22.

Serum biochemical values of the examined heifers on each sampling day are presented in Table 3 and 4. Serum activities of LCAT (Fig. 1) in the diseased heifers were significantly decreased during the acute phase (day 3) compared with those of the postconvalescent phase at day 50. After that, the activities gradually increased and became

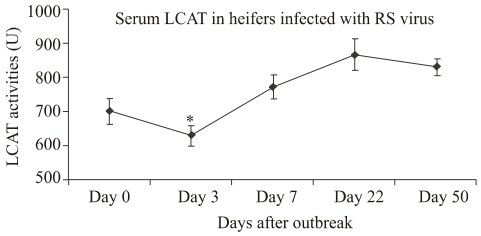

Fig. 1: Serum LCAT in infected dairy heifers.

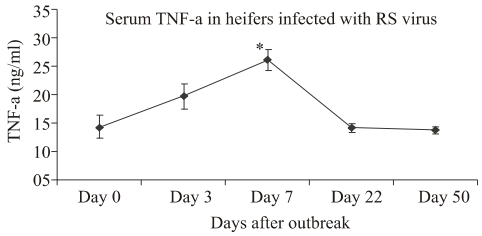

Fig. 2: Serum TNF-α in infected dairy heifers.

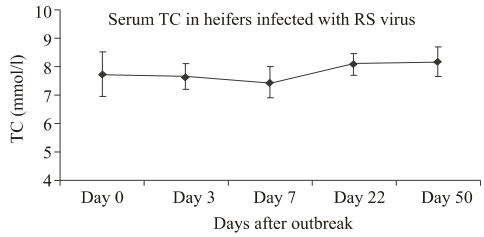

Fig. 3: Serum TC in infected dairy heifers.

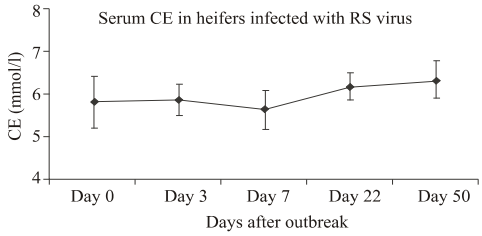

Fig. 4: Serum CE in infected dairy heifers.

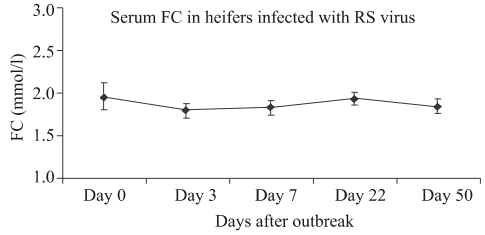

Fig. 5: Serum FC in infected dairy heifers.

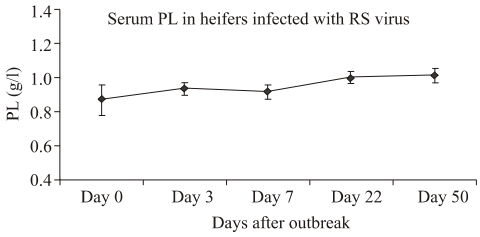

Fig. 6: Serum PL in infected dairy heifers.

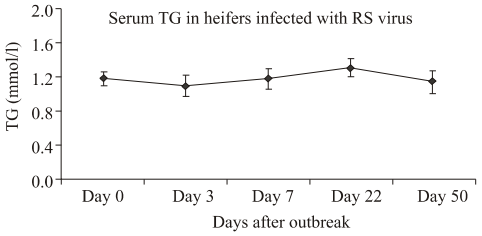

Fig. 7: Serum TG in infected dairy heifers.

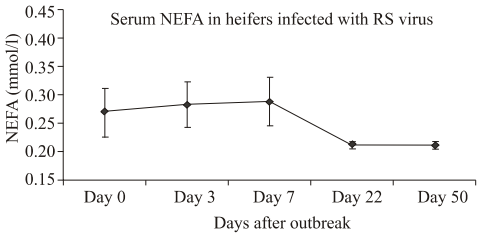

Fig. 8: Serum NEFA in infected dairy heifers.

similar to the postconvalescent phase. Serum concentrations of TNF-α (Fig. 2) increased from day 3 and reached the maximum at day 7. The concentration at day 22 was similar to that at day 50.

Serum concentrations of TC (Fig. 3), CE (Fig. 4) and PL (Fig. 6) were slightly lower during the acute phase compared with the postconvalescent phase, although not significantly. On the other hand, serum concentrations of FC (Fig. 5), TG (Fig. 7) and NEFA (Fig. 8) showed no changes during the acute phase like the three above-mentioned variables.

The serum apoA-I concentration (Fig. 9) was significantly increased on day 0 compared with day 50. After the beginning of therapy, it started to decrease and became similar to the post convalescent value, like acute phase proteins.

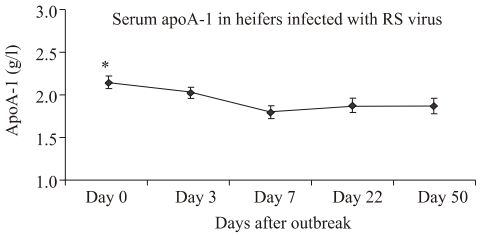

Fig. 9: Serum apoA-I in infected dairy heifers.

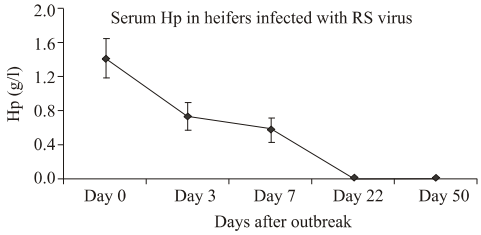

Fig. 10: Serum Hp in infected dairy heifers.

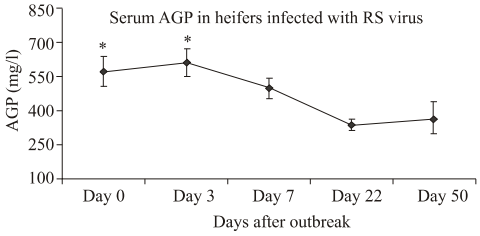

Fig. 11: Serum AGP in infected dairy heifers.

Serum concentrations of Hp (Fig. 10) and AGP (Fig. 11) were remarkably increased in the acute phase (days 0 and 3) compared with the postconvalescent phase (day 50). After the acute phase, the concentration of AGP returned to normal and Hp was undetected.

This study reported that BRSV is considered as a large contributor to or the most common cause of viral respiratory infection in heifers-cows and that agreed with what reported by Baker *et al*. (1986), Larsen (2000), Jim (2009). *Pasteurella multocida* was the most common bacterium isolated from the lavage fluid of calves; *Mannheimia haemolytica* (formerly Pasteurella haemolytica) was not detected (Nikunen *et al*., 2007). According to other studies, *P. multocida* and *M. haemolytica* are the predominant bacteria associated with BRD (Dowling *et al*., 2002).

Incidence of BRSV during an outbreak of respiratory tract disease in our study was 66.67%. This incidence matched with what reported by Baker *et al*. (1986). Collins *et al*. (1988) reported that seropositive rates of 95% in feedlot cows associated with a lack of respiratory disease. Smith *et al*. (1975) mentioned that a serologic survey of Iowa cattle in the 1970s indicated 81% of cattle from 43 herds had neutralizing antibody to BRSV. Gershwin (2007) stated that the etiological factor of enzootic pneumonia in young dairy calves and summer pneumonia in nursing beef calves is a BRSV. In fact, worldwide estimates suggest the frequency of BRSV infections in some dairy and beef herds exceeds 50%. Orro *et al*. (2011) also stated that the initial cause of the enzootic pneumonia in calves was mainly BRSV. Nikunen *et al*. (2007) reported in calves with respiratory diseases that seroconversion to viruses Bovine adenovirus 7 (BAV-7), BAV-3, BCV, BPIV-3 and BRSV was observed in 22.6%, 13.1%, 4.8, 3.6 and 0% of calves, respectively.

The current study mentioned that the diseased heifers became vulnerable to different clinical and blood biochemical changes during the course of respiratory infection with BRSV starting from the onset of the clinical findings up to recovery. The clinical manifestations of the respiratory infection related to RS virus mainly agreed with previous reports (Smith *et al*., 1975; Elazhary *et al*., 1980; Gershwin, 2007; Orro *et al*., 2011) and included cough, nasal discharges, fever, abnormal lung sounds, reduced or complete ruminal atony, anorexia and sometimes dehydration with ocular secretions.

Some studies also reported varying degrees of disease severity have produced due to experimental inoculation with BRSV. Three of 5 calves developed fevers of 40^0C with increase respiratory rates, anorexia, serous nasal discharge, dry muzzle, and malaise (Smith *et al*., 1975).

Diseased heifers infected with RS virus in this study showed blood biochemical changes during the course of infection which included mainly LCAT activities and concentration of TNF-α, Hp and apoA-I. Here, these changes were monitored for the first time in naturally infected heifers with RS virus.

Serum LCAT activities were significantly decreased in diseased heifers at day 3 (BRSV infected heifers). This result was in agreement with previous report (Nakagawa and Katoh, 1999; 2001). Briefly, LCAT activity is reduced in experimental (Nakagawa and Katoh 1999) and natural cases (Nakagawa and Katoh, 2001) of calf pneumonia, suggesting the involvement of cytokines in its expression. However, serum LCAT activity significantly decreased in this study, there were no significant changes in serum concentrations of TC, CE and PL but a slight reduction of their serum concentrations was observed. These results agreed with the previous studies that reported that CE concentrations in the HDL fractions were distinctly decreased in association with reduction of blood activities of LCAT in experimental cases of calf pneumonia, whereas those in the LDL fractions were practically unchanged (Nakagawa and Katoh, 1999). A reduction in LCAT activity with a concomitant depletion of CE, the product of the LCAT reaction, has been reported in lipopolysaccharide-administered African green monkeys (Auerbach and Parks, 1989) Serum concentrations of NEFA, TG, TC, CE and FC were not changed during the course of acute phase. The other studies reported that the FC concentration was firstly raised and thereafter reduced in both bacteria- and virus inoculated groups in experimental cases of pneumonia. Both of FC and PL Concentrations are regulated by complicated metabolic networks. The mechanistic basis of the effects of bacterial and viral inoculations on FC and PL concentrations is still unknown (Nakagawa and Katoh, 1999). It was reported through a study on calves experimentally inoculated with Pasteurella haemolytica and BHV1 that an increase in the blood TG concentration was not reported in both bacteria- and virus inoculated groups in experimental cases of pneumonia. Rather, the TG concentration was decreased in both cases of pneumonia (Nakagawa and Katoh, 1999). One possible explanation for the lowering of blood TG is that amounts of TG taken up by the lung are more than amounts secreted by the liver. The ability of the ruminant to secrete Very Low-Density Lipoproteins (VLDL) is known to be extremely lower than in that in laboratory animals (Pullen *et al*., 1990). Although the current study reported significant elevation in serum TNF in the beginning of infection, the serum TG showed no significant changes and remained within the physiological reference values reported by Radostits *et al*. (2000) [0-14 mmol/l] or by Morrow *et al*. (1979) [0.113±0.09 mmol/l]. In contrast, the previous literatures mentioned that hypertriglyceridemia is reported during the acute states of inflammatory process and cytokines such as TNF-α and interleukin 1b (IL-lb), among others, are thought to mediate the effects of inflammation on metabolism of triglyceride (Price *et al*., 1986; Sherry and Cerami, 1988). TNF suppress lipoprotein lipase synthesis in cell culture systems and several animal species, and also increases hepatic synthesis of triglyceride in rats, both of which may result in hypertriglyceridemia (Sherry and Cerami, 1988; Feingold and Grunfeld, 1987).

On the other hand, previous reports suggested a possible relationship between serum LCAT and TNF-α, but this relationship was unclear. The current study has stated a relationship between significantly reduced serum LCAT activity and

significantly elevated serum TNF- α in dairy heifers naturally infected with BRSV as serum concentration of TNF-α remarkably increased at acute phase (day 7), then decreased gradually until recovery at day 50. The other studies mentioned that lipopolysaccharide (LPS) and TNF-α decrease the plasma LCAT activities (Ettinger *et al*., 1990). Briefly, TNF-α inhibit synthesis of mRNA of LCAT; the production of this enzyme by the liver is reduced. A reduction in LCAT activity with a concurrent depletion of CE has been reported in lipopolysaccharide-administered African green monkeys (Auerbach and Parks, 1989). TNF caused changes in lipoprotein metabolism and enzyme activity similar to those seen with LPS. This suggests that TNF is an important mediator of the observed changes in lipoprotein metabolism after LPS injection in the nonhuman primate (Ettinger *et al*., 1990).

The current results reported that serum LCAT activities appeared to be one of the indicators for diagnosis of naturally occurring respiratory infection in heifers which agreed with previous report mentioned by Nakagawa and Katoh (1999) who said that LCAT activity was practically unaffected by stimuli other than the inoculations. Evaluation of LCAT activity and related lipid concentrations i.e. CE in natural cases in a large sample size is clearly required to assess value of the LCAT for diagnosis of pneumonia in calves.

Serum concentrations of the two APPs i.e., HP and AGP were highly increased at day 0 compared with those of the recovered day (day 50). These findings were considered to reflect the severity of BRSV infection in dairy heifers. However, in the convalecent phase serum Hp was undetected while serum concentration of AGP returned to normal. This may indicate decline the curve of inflammatory reactions as the animals started to recover. Other studies revealed that a stress-induced increase of the serum Hp concentration, independent of respiratory tract disease, has been reported in feedlot cattle (Young *et al*., 1996). This supports the results obtained by the experimental studies by Nakagawa and Katoh (1999) and finally concluded that Hp as acute-phase proteins is produced in response to various stimuli such as stress, and not related only to infection.

On the other hand, experimental infections studies reported that some APPs used as diagnostic markers of respiratory infections in calves after viral (Heegaard *et al*., 2000; Grell *et al*., 2005), bacterial (Schroedl *et al*., 2001; Dowling *et al*., 2004) or combined infections (Ganheim *et al*., 2003). However, only limited data are available on APPs as disease markers of spontaneous BRD (Orro *et al*., 2011).

Hp was considered useful for identifying beef calves with BRD needing treatment and for monitoring treatment efficacy (Carter *et al*., 2002; Humblet *et al*., 2004), whereas AGP was not reported to be a diagnostic marker of BRD in feedlot calves (Carter *et al*., 2002; Berry *et al*., 2004). Orro *et al*. (2011) reported that the initial cause of pneumonia in calves was mainly BRS virus. Respiratory disease caused elevation of APPs in the majority of the calves. The first inflammatory reaction reflected an increase in serum amyloid A (apoSAA) and lipopolysaccharide binding protein (LBP) concentrations at week 1, may be as a response to BRS virus. The subsequent more pronounced elevation of aposaa, LBP and especially Hp is probably due to a secondary bacterial infection. On the other hand, the natural respiratory infections in calves (Nikunena *et al*., 2007) and experimental studies on calves which inoculated *with P. multocida* (Dowling *et al*., 2002) caused respiratory disorders symptoms and elevated serum Hp and AGP concentrations.

Serum concentrations of apoA-I were significantly increased in day 0 compared with that of day 50. The previous studies mentioned that the

apoA-I concentration was increased in a calf with hyperlipidemia compared with healthy controls (Yamamoto *et al*., 2000) and apoA-I was detected with Hp in chylomicrons (CM) and HDL fractions, which suggests the affinity with Hp. The detection of annexins in bronchoalveolar lavage fluids from calves with experimental pneumonia (Katoh *et al*., 1999) may refer to the affinity of apoA-I to annexins, and this because of the association of HDL lipids with the pulmonary surfactants synthesis (Voyno-Yasenetskaya *et al*., 1993).

Some studies reported the association between serum concentrations of ApoA-I and Hp in respiratory infected cows, but there were no evident results. This current study demonstrated a strong association of the elevated serum concentrations of apoA-I, AGP and Hp in naturally viral infected respiratory disorders in heifers where they were concomitantly elevated at the onset of clinical signs and they started to decreased gradually after therapy administration. These results relatively agreed with Voyno-Yasenetskaya *et al*. (1993), Katoh *et al*. (1999), Yamamoto *et al*. (2000).

CONCLUSION

BRSV virus is considered as a large contributor to or the most common cause of viral respiratory infection in heifers-cows. Incidence of BRSV during an outbreak of respiratory tract disease in our study was 66.67%.

Serum LCAT activity was suggested to be one of the indicators for diagnosis of naturally occurring respiratory infection. The changes of serum LCAT activity in BRD complex is involved with the changes of TNF-α, apoA-I, Hp and AGP in the pathogenesis of this inflammation. Reduced serum activities of LCAT were associated with elevated concentrations of serum TNF-α. Serum concentrations of apoA-I are increased concurrently with those of Hp in clinically infected heifers with RS virus, during the inflammatory process. The other reports found that apoA-I acts as a catalyst for the action of LCAT while TNF-α acts as an inhibitory factor for synthesis of LCAT mRNA.

Owing to the previously mentioned observations, the current study concludes that the inhibitory action of TNF-α on LCAT was superior to the catalytic effect of apoA-I. Therefore, serum activity of LCAT significantly decreased in diseased heifers. APPs particularly Hp and AGP, LCAT and TNF-α are sensitive biomarkers of respiratory infection in dairy heifers particularly those with BRSV infection.

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