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Acute Phase Proteins, Lipid Profile and Proinflammatory Cytokines in Healthy and Bronchopneumonic Water Buffalo Calves

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ABSTRACT

The aim of the present study was to evaluate the diagnostic value of Acute Phase Proteins (APP), lipid profiles and proinflammatory cytokines in healthy and bronchopneumonic water buffalo calves. Therefore, sixty water buffalo calves (9±1 month old, 175±15 kg) were divided into two equal groups, the first group represented healthy, control, calves whereas calves of the second group were affected with bronchopneumonia. Total leukocytic and differential counts were determined. Serum total protein, albumin, Triacylglyceol (TAG), low density lipoprotein cholesterol (LDL-c), High Density Lipoprotein cholesterol (HDL-c), Total cholesterol, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), Fibrinogen (Fb), Haptaglobin (Hp), Serum Amyloid A (SAA), Tumor Necrosis Factor-alpha (TNF-α), Interleukins (IL1β, IL-12) and Interferon-gamma (IFN-γ) were also determined. In addition, Bronchoalveolar Lavage (BAL) was collected and analyzed. The present findings indicated that, total leukocytic and neutrophils counts were significantly ($p<0.05$) higher in pneumonic water buffalo calves compare with control. The examined biochemical parameters were significantly ($p<0.05$) increased in pneumonic calves except for total protein, albumin, cholesterol and HDL-c which were significantly ($p<0.05$) lower compare with control. Serum concentrations of investigated APP and proinflammatory cytokines were significantly ($p<0.05$) higher in pneumonic water buffalo calves than those of control. The present study demonstrated that, APP, lipid profile and proinflammatory cytokines perhaps served as biomarkers of bronchopneumonia in water buffalo calves. However, future studies with higher baseline sampling are still needed to establish and validate reference values for APP and cytokines in water buffalo calves.

Keywords: Haptoglobin, Fibrinogen, Serum Amyloid A, Interleukins, Biochemistry, Calves

1. INTRODUCTION

Respiratory diseases in bovine defined as an interaction between environmental, stressful and infectious agents (Galyean *et al*., 1999). Inflammation induced primarily by viral, bacterial, fungal and/or environmental factors (Wilkins, 2003; Wright *et al*., 2010). The primary reason of inflammation can determine the type of cellular response. Changing the concentrations of APP is an early, highly complex reaction of animal body against injurious stimuli (Gabay and Kushner, 1999; Gruys *et al*., 2005a) as a trial to homeostasis and restrains the microbial growth before developing acquired immunity (Gordon *et al*., 2008). APP are of positive (up-regulated; Hp, Fb and SAA) or negative

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(down-regulated; albumin, transferring and α-fetoproteins) response to the challenge (Loughmiller *et al*., 2007; Gabay and Kushner, 1999). This response is accompanied by alterations in lipid metabolism in the form of higher serum triglycerides and lower HDL levels (Cabana *et al*., 1989). Neutrophils in lungs respond to acute inflammation with a series of reactions ended by pathogen destruction (Soethout *et al*., 2002). These reactions involved phagocytizing pathogens, increasing antibodies, complement fixation and finally stimulate production of reactive oxygen and nitrogen (Simms and D'Amico, 1994; Segal, 2005). Moreover, Monocytes and macrophages produced proinflammatory cytokines (TNF-α, IL1β, IL-12 and IFN-γ), mediating the effect of APP, favoring T-helper cell differentiation that construct abridge between innate resistance and adaptive immunity (Guo and Ward, 2002; Trinchieri, 2003). Data concerning APP, lipid profile and proinflammatory cytokines either in clinically healthy or pneumonic water buffalo calves are lacking. Therefore, the objective of the present study was to evaluate the diagnostic values of these biomarkers in buffalo calves.

2. MATERIALS AND METHODS

2.1. Animals and Sampling Protocol

Sixty water Sixty water buffalo calves (9 ± 1 month old, 175 ± 15 kg) were divided into two equal groups, the first group represented healthy, control, calves whereas calves of the second group were affected with bronchopneumonia caused by a transportation journey. The pneumonic calves showed anorexia, coughing, nasal discharge, fever and abnormal lung sound. Diagnosis based on the anamnesis, auscultation and physical examination. Blood samples were collected from the jugular vein into plain and EDTA vacationers from control and pneumonic water buffalo calves. These blood samples were collected after the onset of clinical signs which appeared 2 days post a transportation journey. Whole blood was used for the determination of total leukocytic and differential counts. Sera were harvested and stored at -20°C (Schalm *et al*., 1975) until assayed for total protein, albumin, TAG, LDL-c, HDL-c, Total cholesterol, ALT, AST, ALP, Fb, Hp, SAA, TNF-α, IL-1β and INF-γ. In addition, BAL fluid was obtained from the pneumonic calves by the Tran-tracheal aspiration method (Howard and Smith, 1999).

2.2. Analysis of the Samples

TLC and differential leucocytic counts were determined using electronic cell counter (VetScan HM5 Hematology system). Serum total protein, albumin, TAG, LDL-c, HDL-c and total cholesterol were determined according to the method described by Henry (1966); Doumas *et al*., 1981; Fossati and Prencipe (1982); Friedwald *et al*. (1972); Demacker *et al*. (1980) and Richmound (1973), respectively. VLDL-c was calculated by division of TAG/5 mg dL^{-1} (Bauer, 1982). In addition, serum ALT, AST and ALP enzymes were measured according to the methods described by Bergmeyer and Harder (1986); Kachmar and Moss (1987) and Varley *et al*. (1980), respectively. Fb concentration in plasma was measured with a commercially available ELISA kit (USCA, Life Science) according to the manufacturer's instructions. Serum Hp was measured with a commercially available ELISA kit (Phase SAA kit, Tridelta Ltd., Ireland). SAA was measured with a commercially available ELISA kit (Phase SAA kit, Tridelta Ltd., Ireland), according to the manufacturer's instructions. IL-1β, TNF-α and IFN-γ levels were determined from undiluted serum samples using commercially available ELISA Kits (DIAsource, Diagnostic Corporation, Belgium). The plates were read at 450 nm on a computerized automated microplate ELISA reader (Bio TEC, ELX800G, USA). Values expressed in picograms per millilitre (pg/mL) were extrapolated using linear regression from a standard curve of known amounts of human cytokines. BAL was examined cytologically and bacteriologically. BAL samples obtained from pneumonic water buffalo calves were evaluated for the presence of intracellular organisms. The bacterial isolate were confirmed with Gram staining procedure for intracellular organisms. Bacteriological confirmation was also performed through bronchoalveolar lavage. For bacteriological testing, the BAL samples were diluted 1:10. The volume of 10 µL of each diluted sample was plated onto 7% sheep blood agar and MacConkey agar and was incubated for 24 h at 37°C in 5% CO2 atmosphere (Konewan *et al*., 1992). The bacteria were identified according to the routine procedures (Murray, 1999).

2.3. Statistical Analysis

All data was presented as mean ± standard error of mean by using student-t-test. All tests were performed using computer package of the statistical analysis system (SAS, 2002).

3. RESULTS

Clinical investigation of pneumonic buffalo-calves showed anorexia, coughing, nasal discharge, fever and abnormal lung sound. All pneumonic calves were characterized by similar condition/level of infection and severity. The present findings (**Table 1**) showed significant ($p<0.05$) higher values of TLC and neutrophils in pneumonic calves compared with the control.

Table 1. Hematological parameters in control (n = 30) and pneumonic (n = 30) water buffalo calves

Variables	Control calves	Pneumonic calves
TLC (10^3/mm^3)	8.4±0.18	15.4±0.32*
Neutrophils (%)	29.6±0.52	52.8±2.78*
Lymphocytes (%)	55.7±1.08	55.5±1.09
Esinophils (%)	1.9±0.06	1.9±0.07
Basophils (%)	0.6±0.04	0.6±0.03
Monocytes (%)	2.5±0.02	2.4±0.04

*Means are significantly different at the level (p<0.05)

Table 2. Hepatic and lipid profiles in control (n = 30) and pneumonic (n = 30) water buffalo calves

Variables	Control calves	Pneumonic calves
AST (U/l)	105.8±0.64	292.0±4.88*
ALT (U/l)	88.2±0.46	239.3±9.95*
ALP (U/l)	179.8±1.57	269.0±5.30*
Total proteins (g/dL)	6.4±0.02	5.1±0.02*
Albumin (g/dL)	3.5±0.02	2.5±0.02*
TG (mg/dL)	30.2±0.73	38.8±0.57*
Cholesterol (mg/dL)	60.0±1.60	37.5±0.32*
HDL-c (mg/dL)	26.3±0.26	17.7±0.17*
LDL-c (mg/dL)	22.4±0.67	18.9±0.89*
VLDL-c (mg/dL)	6.1±0.50	7.8±0.30*

*Means are significantly different at the level (p<0.05)

Table 3. Acute phase proteins and Proinflamatory Cytokines in control (n = 30) and pneumonic (n = 30) water buffalo calves

Variables	Control calves	Pneumonic calves
Haptoglobin (g/L)	0.096±0.01	1.18±0.19*
Serum amyloid A (mg/L)	23.9±0.56	166.58±31.48*
Fibrinogen (g/L)	4.2±0.16	16.17±1.18*
TNF-α (ng/mL)	0.42±0.14	2.55±0.12*
IL-1β (pg/mL)	102.43±2.45	640.43±20.45*
IL-12 (ng/mL)	7.45±0.67	25.34±1.43*
IFN-γ (pg/mL)	54.76±1.56	133.65±5.67*

*Means are significantly different at the level (p<0.05)

Furthermore, the values of TG, LDL-c, VLDL-c, AST, ALT and ALP were significantly (P<0.05) higher in pneumonic water buffalo calves when compared with control calves (**Table 2**). However, values of total protein, albumin, total cholesterol and HDL-c were significantly (P<0.05) lower in pneumonic calves than the control (**Table 2**). The current study (**Table 3**) reported significant (P<0.05) higher values of examined APP (Hp, SAA and Fb) and proinflammatory cytokines (TNF-α, IL-1ß, IL-12 and INF-γ) in the pneumonic calves compared with control. Bacteriological BAL culture revealed a presence of predominant 4 classes of microorganisms shared in induction of pneumonia in water buffalo calves. These microorganisms were *Pasteurella* sp., (40%), *Klebsiella* sp. (20%), *E. coli* (13.3%) and finally mixed bacterial infection represented 26.7%.

4. DISCUSSION

The current study considered as one of the first in the field to address water buffalo bronchopneumonia by using APP, lipid profile and proinflammatory cytokines as Biomarkers. The significant (p<0.05) elevation of total leucocytic and neutrophils counts in pneumonic water buffalo calves (**Table 1**) may be attributed to a variety of immunomodulatory effects (El-Ghmati *et al*., 1996). Previous studies (Howard and Smith, 1999; Soethout *et al*., 2002; Civelek *et al*., 2007) reported such increase in leucocytes in pneumonic calves. Moreover, the pathological leucocytes range was reported in several infectious diseases (Kuchler *et al*., 1976; LaMonica *et al*., 1981; Civelek *et al*., 2007). The significant (p<0.05) elevation of ALT, AST and ALP activities and decreased liver albumin production in pneumonic water buffalo calves (**Table 2**) may be associated with possible hepatocellular dysfunction induced by inflammation (bronchopneumonia). Similar higher levels of ALT, AST and ALP were reported by Nikolic *et al*. (2006) in rats and Civelek *et al*. (2007) in neonatal calves. The effect of inflammation on hepatic albumin biosynthesis remains controversial (O'Leary *et al*., 2003). However, lower albumin level observed in the current study agrees with previous findings reported by Civelek *et al*. (2007). Similar marked reduction (p<0.05) of total cholesterol and HDL-c levels, accompanied by significant elevation (p<0.05) of VLDL-c and triglycerides of bronchopneumonic calves were observed in septic patients (Amersfoort *et al*., 2003; Fraunberger *et al*., 1999) and neonatal calves (Civelek *et al*., 2007). The reduction in serum cholesterol in pneumonic calves may be attributed either to inflammatory processes and subsequent changes in lipoprotein metabolism or liver dysfunction (Civelek *et al*., 2007). Lower level of HDL-c perhaps attributed to its protective effects against inflammation which mediated via bacterial endotoxines binding and subsequent neutralization (Wu *et al*., 2004). It was confirmed that inflammation leads to hypertriglyceridaemia in both humans and animals (Alvarez and Ramos, 1986; Phetteplace *et al*., 2000). This may be due to an increased production of VLDL-c, diminished conversion of VLDL-c to LDL-c by the inhibition of lipoprotein lipase activity (Gouni *et al*., 1993) or stimulation of hepatic and adipose tissue lipolysis as well as hepatic fatty acid synthesis, which serve as substrates for hepatic VLDL synthesis (Feingold *et al*., 1992). Hp is α2-globulin synthesized in the liver (Feldman *et al*., 2000) and considered as the

major APP in ruminants. Hp could be detected in infected animals before the onset of clinical signs and that its concentration used as an indicator of disease severity (Godson *et al*., 1996). The increased levels of Hp in cattle interpreted as the outcome of tissue damage resulting from infection or inflammation (Eckersall *et al*., 1988). Similar results in cattle (Conner *et al*., 1986; Eckersall *et al*., 1988; Skinner *et al*., 1991; Saini and Webert, 1991; Heegard *et al*., 2000; Ganheim *et al*., 2003) reported the same significant ($p<0.05$) higher levels of Hp of the current study (**Table 3**). In the contrary, other findings demonstrated either lower (Young *et al*., 1996) or unaltered (Wittum *et al*., 1996) Hp values of infected cows. The significant ($p<0.05$) elevation of SAA of pneumonic water buffalo calves (**Table 3**) perhaps attributed to the physiological role of SAA in host defense during inflammation (Urieli-Shoval *et al*., 2000; Murata *et al*., 2004; Orro *et al*., 2011). Recently, similar marked elevation ($p<0.05$) of SAA was reported in pneumonic calves (Nikunen *et al*., 2007; Orro *et al*., 2011). Fb is a reliable indicator of inflammation and/or bacterial infection in cattle and sheep (Pfeffer *et al*., 1993; Cheryk *et al*., 1998; Hirvonen and Pyorala, 1998; Nikunen *et al*., 2007; Gonzalez *et al*., 2008; Orro *et al*., 2011). The significant ($p<0.05$) elevation of Fb shown in the current study may be attributed to the involvement of Fb in homeostasis, providing a substrate for fibrin formation and in tissue repair, providing a matrix for the migration of inflammatory related cells (Thomas, 2000). Previous research demonstrated elevation of Fb in infected calves (Nikunen *et al*., 2007). Since blood samples were collected one time, the time course of APP, Lipids and cytokines were not estimated. However, it well known that, the acute phase proteins as SAA increased greater than 1000 fold in concentration within 48-72hours following inflammation in man and rabbits (Kushner, 1993). The mechanism for stimulation of APP production is by proinflammatory cytokines. The cytokine groups are the primary inducers of APP gene expression and each type initiates a different pattern of APP (Baumann and Gauldie, 1994). Thus, the increase in different patterns of APP seen in the current study reflected the production of different amounts or types of cytokines (**Table 3**). Similar results concerning such elevated level of TNF-α, IL1β and IFN-γ in inflammation and infection were observed in pigs (Reeth and Nauwynck, 2000) and in cattle (Pace *et al*., 1993; Horadagoda *et al*., 1994; Yoo *et al*., 1995; Knott *et al*., 1998; Morsey *et al*., 1999; Gruys *et al*., 2005b). In addition, expression of TNF and IL were significantly increased in the airways and lung lesions of infected calves (Malazdrewich *et al*., 2001).

5. CONCLUSION

The study demonstrated that, APP, lipid profile and proinflammatory cytokines perhaps served as biomarkers for the diagnosis of bronchopneumonia in water buffalo calves. However, future studies with higher baseline sampling are still needed to establish and validate reference values for APP and cytokines in water buffalo calves.

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6.1. Competing Interests

The researchers declare that they have no competing interests.

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