



Relevance of species-specific procalcitonin (PCT) and C-reactive protein (CRP) as clinical biomarkers in goat pneumonia

AYUSHI S NAIR¹, SARITA DEVI^{1✉}, SUSHIL K MOHAPATRA¹, RATN D SINGH¹, ABHINAV N SUTHAR¹, ANKIT S PRAJAPATI¹, BHAVESH I PRAJAPATI¹ and RAMESH M PATEL¹

College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar, Gujarat 385 506 India

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ABSTRACT

Pneumonia is a leading cause of economic losses in the ruminant industry throughout the world. This study aimed to determine the relevance of species-specific procalcitonin (PCT) and C-reactive protein (CRP) as clinical biomarkers in goat pneumonia. Forty-six confirmed cases of goat pneumonia and 10 apparently healthy goats were included in this investigation. Detailed clinical examination involved recording of vital parameters in diseased as well as in apparently healthy goats. The level of PCT and CRP was analyzed in serum samples collected on day 0 and day 5 from all the 46 diseased goats and on day 0 from 10 healthy goats. A significantly higher pre-treatment concentrations of PCT (229.88 ± 38.79 pg/mL) and CRP (44.13 ± 2.72 µg/mL) in diseased goats was observed when compared with the control group. Significant positive correlation was found between PCT and CRP. Both PCT and CRP revealed a high level of discrimination between diseased goats and healthy ones (AUC= 1.0). Significant inhibition was observed in the values of PCT and CRP 5 days post-treatment in goats treated with two different antimicrobial drugs. Moreover, the obtained data showed a high degree of accuracy for both PCT and CRP in predicting the therapeutic response of infected goats at the selected thresholds (AUC = 1.0 and 1.0, respectively). Positive correlation between PCT and CRP with body temperature and heart rate was recorded. Measurement of PCT and CRP with clinical examination might be useful in assessing severity of goat pneumonia.

Keywords: Biomarkers, C-reactive protein, Goat, Pneumonia, Procalcitonin

Pneumonia of bacterial origin is a frequent health problem in small ruminants (Yener *et al.* 2009). Commonly *Pasteurella multocida*, *Mannheimia haemolytica*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bibersteinia trehalosi*, *Mycoplasma ovipneumoniae* and *Mycoplasma arginini* are isolated and identified as the bacterial cause of pneumonia in goats (Clothier *et al.* 2012, Tushar *et al.* 2013, Smith and Sherman 2022). The respiratory diseases stand for 5.6% of the total small ruminant diseases (Hindson and Winter 2008). In India, the burden of infectious and non-infectious diseases, particularly pneumonia in goats, primarily affects poor rural farmers, with limited reports on its incidence. Recently, morbidity rate of 7.1% and mortality rate of 1.1% due to pneumonia amongst the 11,050 screened Ganjam breed of goats from Odisha was documented (Kama *et al.* 2022). Pneumonia is a major cause of mortality in goats in the study area, highlighting the need for timely identification. While advancements in veterinary medicine have introduced biomarkers for infectious disease

diagnosis, their clinical applicability for confirming diseases and evaluating treatment response remains limited as compared to human medicine.

The acute phase proteins (APPs) are a large group of plasma proteins which originate mainly from the liver in response to pro-inflammatory cytokines (IL-1, IL-6, TNF-α) and release at varying concentrations into the bloodstream in response to severity of infection, type of pathogen involved, inflammation, surgical trauma, or stress (El-Deeb and Iacob 2012, Youssef *et al.* 2015). Marked increase in acute phase response (APR) in bacterial infections contrary to viral infections has been reported (Ulutas *et al.* 2011). C-reactive protein (CRP) is reported to be the most used APP exhibiting both pro-inflammatory and anti-inflammatory effects (Pepys and Hirschfield 2003) and is reported to be used to differentiate between bacterial-viral or acute-chronic diseases (Alsemgeest *et al.* 1994). PCT concentration has become a new diagnostic parameter of increasing relevance in identifying infectious process involving systemic inflammatory processes. A precursor to calcitonin, PCT is normally present in low blood concentrations. PCT has also been compared with CRP as a useful clinical diagnostic marker for a bacterial infectious disease (Wacker *et al.* 2013, Masia *et al.* 2017).

The clinical applicability of these biomarkers in goats

Present address: ¹College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar, Gujarat. ✉Corresponding author email: saritadevi@kamdhenuuni.edu.in

in India is poorly documented, with only a few reports available, primarily concerning conditions like contagious caprine pleuropneumonia (CCPP), stress, various stages of pregnancy, and specific pathogens such as *Corynebacterium pseudotuberculosis* from abroad (Akgul *et al.* 2000, Quinton *et al.* 2009, El-Deeb *et al.* 2020). Most research on these biomarkers has focussed on calves, canines, pigs, sheep, and lambs. Thus, this study was carried out to measure PCT and CRP levels in pneumonic goats and healthy controls as potential clinical biomarkers.

MATERIALS AND METHODS

The study was approved by the Institutional Animal Ethics Committee (IAEC), of College (No. 734/GO/Re/SL/03/CPCSEA).

Study rationale: The present study was conducted in Banaskantha (24.085560°N, 72.144234°E) district of Gujarat, India during 2020-21 for a period of 8 months. Total 164 goats aged between 2 months to 5 years old, of both sexes presented with respiratory illness (anorexia, coughing, dyspnea and ocularo-nasal discharge) at Teaching Veterinary Clinical Complex (TVCC), College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar were screened for pneumonia. During this period, ten apparently healthy goats from Livestock Research Station (LRS), Sardarkrushinagar were randomly selected as control group animals.

Amongst the 164 goats, 70 goats were found to be positive for pneumonia, based on detailed clinical examination. Forty-six of 70 pneumonic goats were treated with two different antimicrobial drugs, viz. Marblofloxacin and Cefquinome sulphate for three days and were subjected to biomarker analysis. Whole blood samples were collected by jugular venipuncture into sterile serum clot activator tubes and the serum was then separated by centrifugation at $1,500 \times g$ for 10 min and stored at -20°C for further biochemical analysis. Blood samples ($n=46$) were collected before and after five days from receiving treatment and only on day 0 in control group animals.

Isolation and identification of bacteria: Sterile cotton swabs ($n=70$) inserted deep into nostril through ventral meatus were collected to determine the bacterial cause of pneumonia. Collected nasal swabs were inoculated into nutrient agar and incubated aerobically at 37°C for 24 h. A loopful inoculum from nutrient agar was streaked on brain heart infusion (BHI) agar plate, blood agar plate (blood agar base supplemented with 7% sheep blood agar; Dehydrated, Hi-Media MP1301) and MacConkey's agar plate and were incubated at 37°C aerobically for 24-48 h for further characterization of bacteria (Quinn *et al.* 2004).

Biomarkers assay: Serum concentrations of PCT and CRP were estimated using a commercially available enzyme-linked immunosorbent assay (ELISA) sandwich kit for goat (ELK Biotechnology Co., Ltd., Wuhan) according to the manufacturer's instructions; the final absorbance was measured in a microtiter plate reader at 450 nm wavelength.

Statistical analysis: Data was analyzed with SPSS statistical software program for Windows (version 20.0). The differences between each examined marker in healthy and pneumonic goats and between pre- and post-treatment values were evaluated by performing the non-parametric Wilcoxon-Mann-Whitney test. Pearson correlation coefficients were used to assess the correlation of the parameters/variables. The results were presented using mean $\pm$ standard error (SEM). $P\text{-value} < 0.05$ was considered statistically significant. To assess the accuracy in differentiating the pneumonic goats from healthy goats, a receiver operator characteristic (ROC) curve was applied to each parameter. The area under the curve (AUC) measured the accuracy to differentiate healthy and pneumonic goats. The ROC analysis also produced a range of potential cut-off values, but the value having highest combined sensitivity and specificity was considered as optimum threshold value (Patbandha *et al.* 2016).

RESULTS AND DISCUSSION

Amongst the respiratory problems faced by small ruminants, pneumonia is one of the most found disease and is a leading cause of economic losses in the small ruminant industry throughout the world (Yener *et al.* 2009). It is reported to be the second most frequent cause of mortality in goats followed by diarrhea due to enterotoxaemia (Dohare *et al.* 2013). A combination of multiple etiological agents (viruses, bacteria, and fungi) and stressors (physical and physiological) are responsible for the occurrence of pneumonia in goats. Irrespective of the etiology, remarkable impact of respiratory diseases upon the profitability of farms in a direct and indirect way (Di Provvido *et al.* 2018) as well as welfare of the animals (Bell 2008) is mentioned.

The current study mainly focussed to ascertain the application of acute phase proteins determinants, i.e. PCT and CRP as clinical biomarkers in naturally affected cases of goat pneumonia. Acute phase reactants to the traumatic, infective, and inflammatory states are called acute phase proteins (APPs) and it can be thought of as an early warning system that strengthens the diagnosis and provides more accurate information on the prognosis of infected animals (Ceciliani *et al.* 2012). There is limited information available about the PCT and CRP concentration in goats, despite being an important clinical determinant. In the present study PCT and CRP levels were found significantly ($p \leq 0.01$) higher in goats suffering from pneumonia as compared to apparently healthy goats

Table 1. Acute phase proteins (CRP and PCT) (Mean $\pm$ S.E.) recorded in apparently healthy and pneumonic goats

Acute phase proteins	Control ($n=10$)	Pneumonic goats ($n=46$)
PCT (pg/ml)	55.39 $\pm$ 1.69	229.88 $\pm$ 38.79**
CRP ($\mu\text{g/ml}$)	8.76 $\pm$ 0.53	44.13 $\pm$ 2.72**

**, $P \leq 0.01$; Statistically highly significant (Mann-Whitney U test).

(Table 1). These findings concurred with those reported by El-Deeb *et al.* (2020), where the concentrations of PCT and CRP was upregulated in goats with contagious caprine pleuropneumonia (CCPP) caused by *Mycoplasma capricolum* ssp. *capripneumoniae* (MCCP). Quinton *et al.* (2009) studied acute phase proteins in cases of bacterial pneumonia in goats and found that acute phase proteins were correlated with the severity of the disease; served as important biomarkers and were functionally significant in such cases.

PCT is APP delivered in the thyroid C cells and is responsible for homeostasis of calcium (being a precursor of calcitonin hormone). The production of PCT is due to endotoxins or mediators produced in response to bacterial infections, hence it may be useful in differentiating viral cause from the bacterial one (Schuetz *et al.* 2011). Its role is being emphasized as a new diagnostic parameter for the diagnosis of inflammatory diseases as well as characterizing the true immune response. PCT is a marker of bacterial infections and is found to be elevated in *Mycoplasma pneumoniae* infection, acute malaria, and fungal infections (Neeser *et al.* 2019). The CRP is another acute-phase reactant, measurements of which is used in the diagnosis of bacterial infections. CRP is synthesized by the liver, mainly in response to IL-6, which is produced during infection as well as in many types of inflammation (Ridker 2003). The mean value of CRP in goats infected with *Corynebacterium pseudotuberculosis* is reported in the range of 1.13- 3.47 mg/dL (Akgul *et al.* 2018). The CRP is considered as an important APP and a precious indicator of the acute inflammatory reaction owing to the marked positive concentration in pneumonic cases, particularly in the early stage of inflammation in pneumonic cases of small ruminants (Haligur and Ozmen 2011).

This study recorded the values of PCT and CRP reverting to normal levels '5' days post-treatment (Table 2). Reports on diagnostic accuracy of different APPs in goats infected with contagious caprine pleuropneumonia (El-Deeb *et al.* 2020), calves infected with neonatal sepsis (Akyüz and Gökce 2021) and respiratory disease in calves associated with bacterial pathogens (*Mannheimia haemolytica* and *Histophilus somni*) (El-Deeb *et al.* 2020) is available, but lacks the post-treatment evaluation of the same as a limitation. Likewise, no reference citing the post-treatment values of APPs (PCT and CRP) in goats suffering from pneumonia in field conditions is available.

Clinically, the studied goats demonstrated anorexia, pyrexia, congested eye mucous membrane, coughing, bilateral mucopurulent nasal discharge, bilateral sero-

Table 3. Clinical parameters (Mean±S.E.) recorded in apparently healthy and pneumonic goats

Parameter	Healthy animals (n=10)	Pneumonic goats (n=46)
Temperature (°F)	101.18 ± 0.29	104.20 ± 0.12**
Heart rate (bpm)	102.20 ± 0.35	181.00 ± 0.86**
Respiratory rate (breaths/min)	25.10 ± 0.23	37.42 ± 0.64**
Rumen contraction (no./5 min)	7.30 ± 0.26	3.27 ± 0.08**

**, P≤0.01; Statistically highly significant.

mucoid nasal discharge, unilateral serous nasal discharge, pale mucous membrane, sneezing, and ocular-nasal discharge, suggestive of pneumonia. The physical examination findings recorded statistically significant (P<0.01) increase in the mean value of temperature (104.20±0.12°F), heart rate (181.00±0.86 bpm), respiratory rate (38.95±0.26 breaths/min) and decrease in rumen contraction (7.30±0.26 /min) in pneumonic goats as compared to healthy goats (Table 3). These findings are in concurrence with earlier works (Abdullah *et al.* 2014, Kacar *et al.* 2018, Rawat *et al.* 2019). The clinical consequences observed in the present study might be attributed to the virulence of the bacterial pathogens (opportunistic as well as environmental), their affinity towards the respiratory tract and an attempt of the host immune system to eradicate the causative microorganisms.

Microbiological examination of nasal swabs collected from infected goats showing apparent clinical respiratory symptoms revealed *Staphylococcus spp.* (n=29), followed by *Pasteurella spp.* (n=20), mixed infection (n=11) and *Bacillus spp.* (n=10) (Table 4). Almost similar findings were reported by Ozbey and Muz (2004) Clothier *et al.* (2012) and Smith and Sherman (2022).

The correlation of PCT and CRP with the clinical parameters in the study and in pneumonic goat's pre and 5-days post-treatment is presented in Table 5. Significant positive correlation (P<0.01) was found between PCT and CRP. Furthermore, a positive correlation of PCT (P<0.05) and CRP (P<0.01) with body temperature and heart rate was observed. Whereas respiratory rate was found to be positively correlated (P<0.01); rumen motility was found to be negatively correlated (P<0.01) with CRP in the present study. The PCT, CRP and body temperature measured 5-days post-treatment showed a negative correlation with pneumonic goats before treatment. Heart rate measured 5-days post-treatment showed a significant (P<0.05) negative correlation with pneumonic goats before treatment.

Table 2. Acute phase proteins (CRP and PCT) (Mean±S.E.) recorded in control and pneumonic goats for two different treatment groups (before and after)

Acute phase proteins	Group I (Control) (n=10)	Group II (n=26)		Group III (n=20)	
		Day 0	Day 5	Day 0	Day 5
PCT (pg/mL)	55.39 ± 1.69 ^a	239.06 ± 55.10 ^b	56.88 ± 1.65 ^a	217.94 ± 54.64 ^b	54.72 ± 1.96 ^a
CRP (µg/mL)	8.76 ± 0.53 ^a	43.75 ± 3.95 ^b	7.95 ± 0.25 ^a	44.63 ± 3.71 ^b	7.50 ± 0.30 ^a

Mean bearing different superscript (a, b) in a row differ significantly, P≤0.01 and P≤0.05.

Table 4. Morphological characteristics of bacteria isolated from pneumonic goats (n=70)

Organism isolated	Morphological characteristics	No. of isolates (n=70)	Percentage (%)
<i>Staphylococcus spp.</i>	Gram +ve, cocci, arranged in grapes like cluster	29	41.42
<i>Pasteurella spp.</i>	Gram -ve, coccobacilli	20	28.57
Mixed infection	Gram +ve, cocci, Gram -ve, coccobacilli+ Gram + ve, rods	11	15.71
<i>Bacillus spp.</i>	Gram + ve, rods	10	14.28

Table 5. Correlation values of PCT and CRP and vital parameters in healthy/pneumonic goats, and in pneumonic goats pre- and 5-days post-treatment

Parameter	PCT (pg/mL)	CRP (µg/mL)	Pre- and post-treatment
PCT (pg/mL)	-	0.370**	-0.219
CRP (µg/mL)	0.370**	-	-0.357
Temperature (°F)	0.301**	0.500**	-0.025
Heart rate (bpm)	0.271*	0.581**	-0.291*
Respiratory rate (breaths/min)	0.259	0.387**	0.044
Rumen contraction (frequency/5 min)	-0.143	-0.576**	0.080

**, P≤0.01: Statistically highly significant; *, P≤0.05: Statistically significant.

While respiratory rate and rumen motility measured 5-days post-treatment showed a non-significant negative correlation with pneumonic goats before treatment. The findings of the present study were indicative of the stress associated with undergoing inflammatory/pathological progress like pneumonia. Likewise, almost similar pattern of recordings with different set of parameters has been reported in calves with sepsis (Akyüz and Gökce 2021).

In this study, the tested biomarker's ability to discriminate pneumonic goats from healthy goats was evaluated with ROC analysis. The accuracy to differentiate healthy and infected goat was 100% (AUC=1.0) for both CRP and PCT. The optimum threshold values for CRP and PCT were 102.9 pg/mL and 14.74 µg/mL, respectively, and the corresponding sensitivity and specificity was 100% (Fig. 1). Estimation of acute phase proteins in plasma or serum has been reported to provide valuable diagnostic information and monitoring of the disease process in several animal species (Eckersall 2000).

The use of PCT and CRP biomarkers in goats with pneumonia is limited, particularly post-treatment. The study found significant changes in serum PCT and CRP levels and vital parameters in pneumonic goats compared to healthy ones. Both biomarkers decreased with treatment, highlighting their potential as clinical indicators. Findings of present study can open a new avenue in the clinical studies in goats about PCT and CRP.

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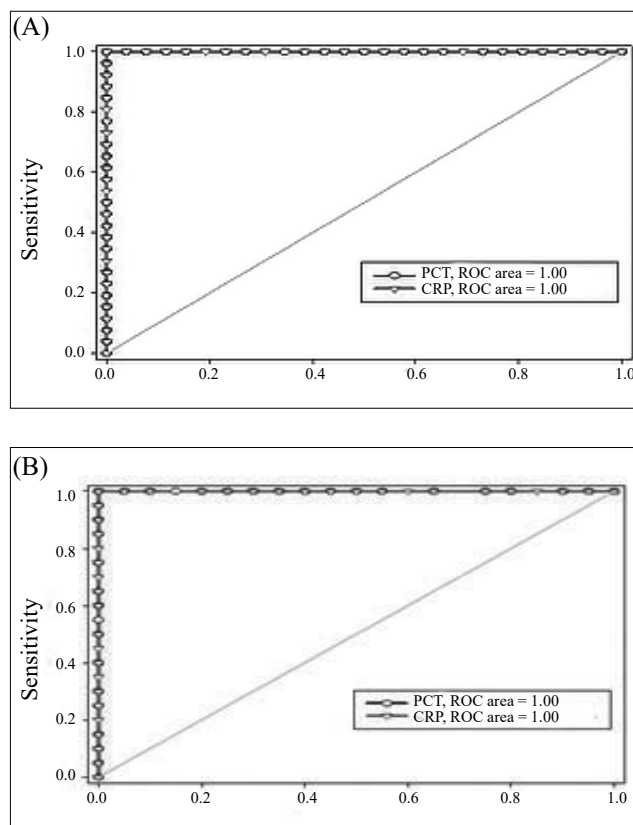


Fig. 1. Receiver operating characteristic (ROC) curve analysis of (A) PCT and (B) CRP.

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