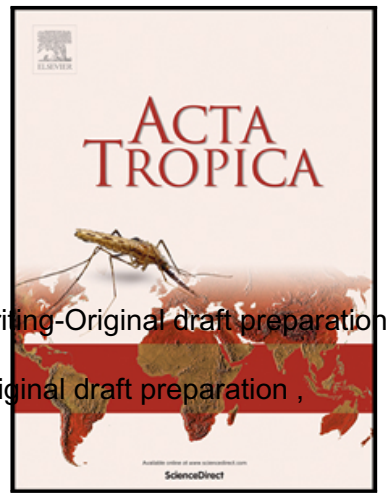

Use of procalcitonin, neopterin, haptoglobin, serum amyloid A and proinflammatory cytokines in diagnosis and prognosis of bovine respiratory disease in feedlot calves under field conditions

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Highlights

- Procalcitonin, neopterin are new biomarkers for respiratory disease in feedlot calves.
- Higher levels of cytokines was detected in feedlots with respiratory disease.
- Haptoglobin and serum amyloid A showed higher levels in diseased feedlot calves.
- Blood biomarkers declared a high level of diagnostic and prognostic accuracy.
- Biomarkers could assist in treatment evaluation in calves with respiratory disease.

Use of procalcitonin, neopterin, haptoglobin, serum amyloid A and proinflammatory cytokines in diagnosis and prognosis of bovine respiratory disease in feedlot calves under field conditions

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Abstract

Bovine respiratory diseases (BRD) have long been considered a serious problem that causes major economic losses in feedlot calves (FC). This study aimed to determine the diagnostic and prognostic effect of selected biological markers including, procalcitonin (PCT), neopterin (NP), proinflammatory cytokines (IL-1β, IL-8, TNF-α, IF-γ), haptoglobin (HP) and serum amyloid A (SAA) on FC with BRD under field conditions. Sixty-nine FC that were identified to be infected with *Mannheimia haemolytica* and *Histophilus somni* and had different clinical respiratory signs (diseased group) were selected for this study. In addition, 20 healthy FC have been selected as a control group. We have detected higher serum levels of PCT, NP, HP, SAA, IL-1β, IL-8, TNF-α, IF-γ, in diseased FC group compared with the control group. All tested markers revealed a high level of discrimination between BRD infected FC and healthy ones (AUC > 0.90). Moreover, the obtained data showed a high degree of prognostic accuracy for PCT, NP, IL-8, HP, IF-γ and IL-1β in predicting treatment response of FC with BRD at the selected thresholds (AUC = 0.99, 0.99, 0.97, 0.93, 0.88 and 0.82 respectively). Significant inhibition was observed for the selected biochemical markers in treated FC 7 days post-treatment. In conclusion, this study showed that BRD in FC was associated with significant alterations in serum APPs, proinflammatory cytokines, PCT and NPT levels. Furthermore, it demonstrated that these serum biomarkers are much higher in FC with BRD compared to recovered ones. Our data suggest that the measurement of PCT, NPT, APPs and cytokines

together with the clinical examination may be a useful diagnostic and prognostic tool for assessment of FC naturally infected with *M. haemolytica* and *H. somni*.

Key words: Procalcitonin, Neopterin, Haptoglobin, Cytokines, Serum amyloid A, Bovine respiratory disease, Feedlots

1. Introduction

Bovine respiratory disease (BRD) is a major problem worldwide, causing a significant reduction in numbers of calves in beef and dairy farms (Arslan, 2008). Several pathogens, comprising *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma* spp., *Histophilus somni*, and *Archanobacter pyogenes* are among the most common causative agents of BRD (Angen et al., 2009; Ceciliani et al., 2012; Başbuğ et al., 2016). However, *H. somni* has not yet been reported as a causative agent of BRD in the Kingdom of Saudi Arabia. Fever, septicemia, hypoxia, secretion of acute phase proteins (APPs) from the hepatic cells, endotoxin and exotoxin production, lung abscesses, and vasculitis may well occur in BRD cases (Başbuğ et al., 2016; de Carvalho et al., 2016) leading to myocardial injury (Dinler et al., 2017). In human medicine, blood biomarkers are regularly used for confirmation of the types of inflammation and the body response to the treatment. However, in animals a very low number of biomarkers are utilized routinely; this could help identify the inflammatory process or evaluate disease outcome.

APPs are used as sensitive biomarkers of inflammation and/or infection (Tothova et al., 2014). APPs delivered in the liver and emitted into the bloodstream because of the challenges with different pathogens or inflammatory conditions (El-Deeb and Iacob, 2012; Youssef et al., 2015). The release of APPs into the blood varies according to the disease severity and the type of pathogens (Petersen et al., 2004; Youssef et al., 2015). In bacterial infections, the acute phase

response (APR) is significantly increased, whereas it is less pronounced during viral infections (Ulutas et al., 2011). Haptoglobin (HP) and serum amyloid A (SAA) are considered the leading APPs that have been studied in bovine species (Schrödl et al., 2016). HP is an α2-globulin and has a bacteriostatic effect through its ability to bind free hemoglobin (Tirziu, 2009). SAA mediates phagocytic cells migration to the infection site and acting as a chemoattractant (Abdallah et al., 2016). Higher serum levels of APPs in farm animals is associated with inflammatory processes, including enteritis, endocarditis, urinary tract infection, pneumonia or peritonitis (El-Deeb and Buczinski, 2015; El-Deeb and Elmoslemany, 2016a; El-Deeb and Elmoslemany; 2016b; El-Deeb et al., 2017).

Proinflammatory cytokines act as messengers between the hepatocytes and the infection site (Jain et al., 2011), induce the synthesis of APPs and APR. Production of interleukin (IL) and tumor necrosis factor α (TNF-α) from the macrophage or monocytes in response to infection, induces synthesis and secretion of APPs from the liver (Jain et al., 2011). The routine clinical diagnosis of respiratory diseases in calves remains challenging and the identification of causative agents is scarce (Joshi et al., 2018). In addition, the detection of antibodies in the calf serum at the onset of infection is difficult due to low sensitivity (de Carvalho et al., 2016). Thus, measurement of serum APPs and proinflammatory cytokines concentrations may potentially be used as an additional diagnostic and prognostic tool for diagnosis and prognosis of BRD.

Procalcitonin (PCT) is an acute phase protein delivered in the thyroid C cells and is a precursor of calcitonin hormone accountable for homeostasis of calcium. PCT is considered as a quantifiable marker in provocative reactions in bacterial and parasitic infections, due to its ability to increase quickly after the production of specialized cytokines (TNF-α, IL-6 and IL-8) (Reinhart et al., 2000). The information about the analytical estimation of PCT is arguable.

Although some studies revealed that PCT is more reliable for analysis of neonatal sepsis than CRP (Guibourdenche et al., 2002; Chiesa et al., 2003; Joram et al., 2006; Naher et al., 2011). Neopterin (NPT) is a low molecular weight biomarker associated with cell-mediated immunity and produced by dynamic monocyte/macrophage (Werner-Felmayer et al., 1990). IFN-γ is a potential NPT trigger that increases the NPT levels in the body fluids (Stang and Koller, 1998).

To the best of our knowledge, there are no data available about the changes in PCT and NPT levels in feedlot calves (FC) naturally infected with *M. haemolytica* and *H. somni*. Therefore, in this study we aimed to measure the APPs, proinflammatory cytokines, PCT and NPT levels in BRD infected FC and those recovered ones and evaluate their accuracy as an additional tool for diagnosis of BRD infected FC and as treatment efficacy biomarkers.

2. Materials and methods

2.1. FC enrollment and sampling

Sudden onset of respiratory manifestation was observed sporadically at a private farm in Al-Kharg region, Saudi Arabia. A total of 840 FC were eligible for enrolment and visual assessment at each farm visit. FC (n = 250) that showed any of the BRD signs, including depression, decreased appetite, nasal and ocular discharge, congested mucous membrane, abnormal respiratory sounds, and dyspnea were considered BRD-suspected cases and marked for sampling between July and August 2016.

Nasopharyngeal swabs and blood samples were collected from each BRD-suspected FC as illustrated in Fig. 1. Nasopharyngeal swabs were collected from each FC using a guarded polyester swab (Culture Swab-Kalayjian, Patterson Veterinary Supply Inc., USA). Swabs were submitted for viral (Bovine Respiratory Syncytial Virus (BRSV), ParaInfluenza 3 (PI3), Bovine

herpesvirus-1 (BHV-1) and Bovine Viral Diarrhea Virus (BVDV)) and bacterial (*P. multocida*, *M. haemolytica*, *H. somni*, *M. bovis*) detection. Whole blood samples were collected by jugular venipuncture into sterile and evacuated tubes that did not contain an anticoagulant and were transported to the laboratory in a cooler at 4 °C. The serum was then separated by centrifugation at 1,500 × g for 10 min, divided into three aliquots and stored at –20 °C for further biochemical analysis. In addition to the BRD-suspected FC, twenty apparently healthy feedlots from the same farm were randomly selected and enrolled as control healthy group. Nasopharyngeal swabs and blood samples were also collected from the control animals to confirm their BRD-negative status and for further biochemical analysis.

A feedlot calf was confirmed as BRD-positive case if 1) it was identified as BRD-suspected case and 2) one or more of BRD viral and/or bacterial infections were detected. BRD confirmed FC (n = 69) were treated with one of the following antibiotics (Draxxin® (tulathromycin), Nuflor® (florfenicol), Pulmotil® (Tilmicosin), and Excenel® (ceftiofur)) and anti-inflammatory Flunixin meglumine and Phenylbutazone (FM and FBZ). The treatment regimen and doses for each drug were selected according to the manufacture's recommendations. Blood samples (n = 51) were collected after seven days from receiving treatment. Samples have not been taken from 18 of the 69 FC due to them being moved to other farms or dead. This investigation was directed in harmony with the Animal Ethics Committee guidelines, King Faisal University, Saudi Arabia (protocol No: 7/B/9512).

2.2. Viral and bacterial pathogens isolation

Nasopharyngeal swabs were analyzed for the presence of viral pathogens (BRSV, PI3, BVDV and BHV-1). Total DNA and RNA were extracted from swabs using QIAamp DNA mini

kits and RNeasy Mini Kit (Qiagen SA, Courtaboeuf, France), respectively. The PCR was performed according to the standards conditions described elsewhere (Horwood and Mahony, 2011; Klima et al., 2014). For bacterial pathogens, nasopharyngeal swabs were inoculated on brain heart infusion agar (Difco, Oxoid Limited, UK) supplemented with 5% sheep blood agar, 0.5% yeast extract (Difco, Oxoid Limited, UK) and 5 mg/mL of bacitracin and Colombia blood agar (Oxoid Limited, UK) supplemented with 5% sheep blood and selective antibiotics as described previously (Slee and Stephens, 1985). All inoculated plates were incubated at 37 °C for 24–48 h at 5% CO_2 enriched environment. Colonies displaying morphology indicative of *H. somni* and *M. haemolytica* were identified using VITEK 2 Compact (BioMérieux, France).

H. somni and *M. haemolytica* recovered from BRD-infected calves were subjected to 16S rRNA sequencing using universal primers: 27F (5′-AGRGTTTGATCMTGGCTCAG) and 1492R (5′-GGTTACCTTGTTACGACTT). PCR conditions used were described previously (Lane, 1991; Holman et al., 2015). All amplified PCR products were purified using the QIAquick PCR purification kit (Qiagen SA, Courtaboeuf, France) according to the manufacturer's instructions and sequenced using a Genetic analyzer 3500 (Applied Biosystems). The 16S rRNA sequence homology searches were carried out using NCBI Basic Local Alignment Search Tool (BLAST).

2.3. Biomarkers analysis

2.3.1. Acute phase proteins

Haptoglobin (HP) and serum amyloid A (SAA) were measured in the serum samples of the FC using commercial test kits (Tridelta Development Ltd., Kildare, Ireland) according to the manufacturer's instructions. The hemoglobin peroxidase activity, which is in direct proportion to

the quantity of HP, was measured by detecting serum HP levels. In addition, the SAA level was determined using a solid sandwich ELISA.

2.3.2. Proinflammatory cytokines

The serum concentrations of proinflammatory cytokines (IL1-β, IFN-γ, IL-8 and TNF-α) were measured using commercial ELISA (CUSABIO Biotech, Wuhan, China) according to the manufacturer's instructions for cattle.

2.3.3. Procalcitonin and neopterin

The serum levels of procalcitonin (PCT) were detected using a commercial test kit for cattle (Bovine Procalcitonin (PCT) ELISA Kit, CUSABIO Biotech, Wuhan, China). However, the serum neopterin (NPT) concentrations were detected using ELISA kits (bovine NPT (Neopterin) ELISA Kit, FineTest, Wuhan Fine Biotech, Wuhan, China) according to the manufacturer's instructions for cattle.

2.4. Statistical analysis

Data were analyzed with Stata statistical software program for Windows (version 15.0; StataCorp, 2017). The differences between each examined marker in healthy and BRD-infected FC and also between pre- and post-treatment were evaluated by performing the Normality and Wilcoxon-Mann-Whitney test. The Spearman's rank correlation coefficients were used to assess the correlation of the parameters.

In order to assess the accuracy in differentiating the BRD-infected FC from healthy calves, a receiver operator characteristic (ROC) curve was applied to each parameter. The area

under the curve (AUC) was calculated. Moreover, the Youden index (= maximum [sensitivity + specificity-1]) was used to identify the optimal cut-off values for detection of BRD-infected FC. Diagnostic and prognostic test characteristics such as sensitivity (Se), specificity (Sp) and accuracy were calculated for each biomarker. The proportion of those FC that were correctly identified as BRD-infected was determined as Se, whereas the correctly identified proportion of healthy calves was determined as Sp and the proportion of correctly classified FC was determined as Accuracy. In addition, based on each biomarker, the level of agreement between calves categorized as healthy or as BRD positive was evaluated by Cohen's kappa statistic (κ).

Results

A total of 840 FC with an average age of 8 months (range: 4 to 12 months) were in the study herd. Of the 840-feedlot calves, 250-feedlot calves were identified as BRD-suspected cases using visual and clinical examinations. Only 69 calves were identified as BRD-infected with *M. haemolytica* and/or *H. somni* (51 calves infected with *M. haemolytica* only, 5 calves with *H. somni* only and 13 calves infected with both). The 16S rRNA gene sequences of each isolate were subjected to BLAST analysis and representative sequences were submitted to GenBank with the following accession numbers: MG725885, MG725884.1, MG725883, MG725828, MG725827, MG725826.1, MG725726. *P. multocida*, *M. bovis*, BRSV, PI3, BHV-1 and BVDV were not identified in any sample using PCR examination. The number of BRD infected FC treated with tulathromycin, florfenicol, Tilmicosin and Ceftiofur were 26, 19, 13 and 11 FC, respectively. However, the number of FC treated with anti-inflammatory FBZ and FM were 26 and 43, respectively.

The serum levels of HP, SAA, proinflammatory cytokines (IL-1β, IFN-γ, IL-8, TNF-α), PCT, and NPT in healthy and BRD positive FC are presented in Fig. 2. The levels of HP, SAA, proinflammatory cytokines, PCT, and NPT were significantly ($P < 0.0001$) higher in BRD infected FC than healthy controls. Figure 3 displays the levels of HP, SAA, proinflammatory cytokines (IL-1β, IFN-γ, IL-8, TNF-α), PCT, and NPT in BRD infected FC pre- and 7 days post-treatment. A significant ($P < 0.0001$) decline occurred in HP, SAA, proinflammatory cytokines, PCT, and NPT concentrations of BRD infected cases within 7 days post-treatment.

Spearman's correlation coefficient was determined for the study biomarkers in BRD infected FC and control and in BRD infected FC pre- and 7 days post-treatment (Table 1). All measured parameters were positively correlated with BRD infected FC. However, all parameters measured 7-days post-treatment showed a negative correlation with BRD infected FC before treatment.

ROCs were generated (Fig. 4) and the optimum cut-off values for each parameter differentiating between BRD infected FC and healthy controls were determined based on the Youden index. The diagnostic test characteristics (Se, Sp, and accuracy) associated with the optimal cut-off values of each parameter are presented in Table 2. The agreement levels between the number of calves designated as healthy or infected with BRD by each parameter were assessed using Cohen's kappa (κ) statistic and presented in Table 2. Moreover, the obtained data showed a high degree of prognostic accuracy for PCT, NP, IL-8, HP, IF-γ, IL-1β, TNF-α and a moderate degree for SAA in predicting treatment response of FC with BRD at the selected thresholds as shown in Table 3 and Fig. 5.

Discussions

BRD is a major cause of morbidity and mortality in both beef and dairy-producing calves (Panciera and Confer, 2010). BRD is multifactorial and caused by the interaction of pathogenic bacteria and viruses. However, in the present study, all confirmed BRD infected FC were infected with *M. haemolytica* and *H. somni* as determined by PCR. This study aimed to scrutinize the blood changes in APPs, proinflammatory cytokines, PCT and NPT in FC naturally infected with *M. haemolytica* and *H. somni* and recovered FC. Furthermore, to evaluate their use as an additional tool for diagnosis and prognosis of BRD infected FC.

In ruminant, HP and SAA are considered the dominant APPs, which increased throughout infections, inflammatory conditions, surgical trauma and stress (Heegaard et al., 2000; Petersen et al., 2004). In this investigation, there is significant elevations in serum HP and SAA levels in BRD infected FC, indicating a strong immune response to the *M. haemolytica* and *H. somni* infections. The elevated HP levels may be a result of cell injury (lung tissues) following the infection (Blackmore, 1988). Serum HP is considered a strong bacteriostatic protein against various bacterial pathogens by restricting free hemoglobin and denying microorganisms from iron required for their development (Eaton et al., 1982). On the other hand, SAA response may be attributed to its significant role in modulating immune defense of animals during infection and/or tissue injury (Urieli-Shoval et al., 2000; Murata et al., 2004; Orro et al., 2011). Moreover, SAA alters cholesterol metabolism during different inflammatory problems (Pannen and Robotham, 1995). Also, it was stated that SAA could inhibit the growth of microorganisms (Hari-Dass et al., 2005), by simplifying the process of phagocytosis of pathogenic bacteria (Larson et al., 2005). Similar findings have been reported in BRD infected calves due to *P. multocida* (Dowling et al., 2002), mixed infection of *Staphylococcus aureus* and *E. coli* (Joshi et al., 2018), buffalo calves with bacterial bronchopneumonia (El-Bahr and El-

Deeb, 2013) and sheep with pneumonic pasteurellosis (El-Deeb and Elmoslemany, 2016a). However, other studies have reported a limited association between respiratory diseases and HP levels in FC (Wittum et al., 1996; Young et al., 1996).

The levels of cytokines (IL-1β, IL-8, TNF-α, IF-γ) in BRD infected FC were much higher than those in healthy controls, indicating that infections with *M. haemolytica* and *H. somni* are associated with solid APR and pathological changes in BRD infected FC. Cytokines play a significant role in BRD pathogenesis in FC and are consistent with those previously reported in *P. haemolytica* infected calves (Pace et al., 1993; Horadagoda et al., 1994; Morsey et al., 1999; Malazdrewich et al., 2001), sheep with pneumonia (El-Deeb and Elmoslemany, 2016a) and *Coxiella burnetii* infected sheep, goats and she-camels (El-Deeb et al., 2019).

Interestingly, FC with BRD showed a significant increase in serum PCT and NPT concentrations than that in healthy control ones involved in this study. PCT increased quickly in circulation in response to inflammation, which resulted from infection and after the production of selected cytokines (TNF-α, IL-6 and IL-8) (Reinhart et al., 2000). Previous studies have been reported that PCT increased than the normal serum level in septicemias (Assicot et al., 1993; Dandona et al., 1994) and bacterial infection cases (Tünger, 2007). Remarkably, the higher serum levels of NPT in this study could trigger cellular immune response in BRD infected FC.

Monocytes/macrophages produce NPT as a result of stimulation by interferon-γ, which is set free from activated T cells. Contrariwise, other body cells do not produce measurable quantities of NPT as a result of various stimuli. Consequently, the production of NPT seems to be closely related to the cellular immune response activation (Werner-Felmayer et al., 1990). One of the most critical functions of neopterin is that it inhibits the folate synthesis by pathogenic intracellular bacteria (Huber et al., 1983). Comparable results have been reported

with different bacterial, viral and parasitic diseases (Shaw, 1991; Facer, 1995) and in calves with septicemic colibacillosis (Ercan et al., 2014). The evident increase in serum APPS, cytokines, PCT and NPT in BRD infected FC than that in recovered ones indicating that these parameters could be used in the evaluation of treatment efficacy. Previous studies have documented the use of these parameters in prediction of recovery in both humans and animals (El-Azeem et al., 2013; Nanda et al., 2016; El-Deeb and Elmoslemany, 2015; El-Deeb and Buczinski, 2015; El-Deeb et al., 2017).

The ROC analysis was used to assess the ability of APPs, cytokines, PCT and NPT to differentiate between BRD infected and heathy control FC. All examined parameters displayed a high level of discrimination between BRD infected FC and healthy ones (AUC > 0.90), which in accord with the diagnostic accuracy guidelines (Swets, 1988). The optimal threshold, which achieved the highest Se, Sp and accuracy, was selected based on the Youden index. Cytokines (IFN-γ, IL-8, and TNF-α), PCT and NPT showed the highest Se, Sp and accuracy and hence, can potentially be considered as an additional diagnostic tool for BRD infected FC. However, NPT is biochemically dormant as its half-life in the body is a result of renal discharge (Hamerlinck, 1999). Consequently, the detection of NPT has several advantages compared to cytokines, which have a short half-life and fast degradation.

Data in this study revealed a high degree of prognostic accuracy for PCT, NP, IL-8, HP, IF-γ, IL-1β, TNF-α and a moderate degree for SAA in predicting treatment response of FC with BRD at the selected thresholds. APPs and cytokines previously used as prognostic indicators for many diseases as it can reflect the treatment efficiency. Moreover, their levels also reflect the severity of the disease condition and the possible degree of tissue injury. Our results are comparable with those reported by Skinner et al. (1991), Heegaard et al. (2000) and Carter et al.

(2002). Alterations in the blood levels of APPs specify the necessity for a more comprehensive clinical assessment of FC with BRD. Moreover, APPs can be considered as a great tool in the monitoring of treatment in diseased FC.

In this study, the use of PCT and NP as prognostic biomarkers in FC with BRD has demonstrated great potential in evaluating disease outcomes. The uses of these new biomarkers in animals is very limited. The aforementioned results agree with those previously reported for assessing the prognosis of sepsis using PCT (Giamarellos-Bourboulis et al., 2002; Baydar et al., 2009; Sharma et al., 2014). A PCT and NP values that obtained at the time of clinical examination of FC with BRD is a predictor disease outcome (high PCT and NP levels are expressively associated with poor prognosis) and thus may identify those BRD cases that have been classified as increased mortality risk, and aid the improvement of the treatment protocols.

Conclusions

The present study showed that BRD in FC was associated with a significant alteration in serum PCT, NPT APPs and proinflammatory cytokines levels. Furthermore, this study demonstrated that these biomarkers are higher in FC with BRD compared to recovered ones. The obtained results propose that the measurement of APPs, cytokines, PCT and NPT, together with the clinical examination of FC may potentially be used as a diagnostic and prognostic tool for assessment of FC naturally infected with *M. haemolytica* and *H. somni*.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1. Correlation among acute phase proteins, proinflammatory cytokines, procalcitonin and neopterin in healthy and bovine respiratory disease (BRD) infected feedlot calves, and in BRD positive calves pre- and 7 days post-treatment.

Parameters[1]	Healthy and diseased			Pre- and post-treatment		
	N	Correlation	*P-value*	N	Correlation	*P-value*
HP (gm/L)	89	0.63	<0.001	102	-0.75	<0.001
SAA (µg/mL)	89	0.64	<0.001	102	-0.46	<0.001
IL-1β (pg/mL)	89	0.61	<0.001	102	-0.56	<0.001
IFN-γ (pg/mL)	89	0.64	<0.001	102	-0.67	<0.001
IL-8 (pg/mL)	89	0.69	<0.001	102	-0.82	<0.001
TNF-α (ng/mL)	89	0.66	<0.001	102	-0.54	<0.001
PCT (pg/mL)	89	0.72	<0.001	102	-0.87	<0.001
NPT (ng/mL)	89	0.72	<0.001	102	-0.87	<0.001

[1] HP = haptoglobin; SAA = serum amyloid A; IL-1β = interleukin 1-beta; IFN-γ = Interferon gamma; IL-8 = interleukin 8; TNF-α = tumor necrosis factor-alpha; PCT = procalcitonin; NPT = neopterin.

Table 2. Diagnostic test characteristics of acute phase proteins, proinflammatory cytokines, procalcitonin and neopterin in healthy and bovine respiratory disease infected feedlot calves.

Parameters[1]	Threshold	Diagnostic characteristics (%)[2]			*J*[3]	K[4]	BRD	Test
		Se (95% CI)	Sp (95% CI)	Accuracy			-/+	-/+
HP (gm/L)	≥0.59	81.2 (69.9-89.6)	100 (83.2-100)	85.4	0.81	0.66	20/69	33/56
SAA (µg/mL)	≥24.36	89.9 (80.2-95.8)	80 (56.3-94.3)	87.6	0.70	0.66	20/69	23/66
IL-1β (pg/mL)	≥107.26	89.9 (80.2-95.8)	90 (68.3-98.8)	89.9	0.80	0.73	20/69	25/64
IFN-γ (pg/mL)	≥55.21	92.8 (83.9-97.6)	95 (75.1-99.9)	93.3	0.88	0.82	20/69	24/65
IL-8 (pg/mL)	≥81.21	97.1 (89.2-99.6)	100 (83.2-100)	97.8	0.97	0.94	20/69	22/67
TNF-α (ng/mL)	≥0.47	94.2 (85.8-98.4)	85 (62.1-96.8)	92.1	0.79	0.78	20/69	21/68
PCT (pg/mL)	≥48.62	100 (94.8-100)	90 (68.3-98.8)	97.8	0.90	0.93	20/69	18/71
NPT (ng/mL)	≥4.21	100 (94.8-100)	90 (68.3-98.8)	97.8	0.90	0.93	20/69	18/71

[1] HP = haptoglobin; SAA = serum amyloid A; IL-1β = interleukin 1-beta; IFN-γ = Interferon gamma; IL-8 = interleukin 8; TNF-α = tumor necrosis factor-alpha; PCT = procalcitonin; NPT = neopterin.

[2] Se = sensitivity; Sp = specificity; accuracy = percentage of correctly classified samples.

[3] *J* = Youden index.

[4] κ = Cohen's kappa value.

Table 3. Prognostic characteristics of acute phase proteins, proinflammatory cytokines, procalcitonin and neopterin in bovine respiratory disease infected and recovered feedlot calves.

Parameters[1]	Threshold	Prognostic characteristics (%)[2]			*J*[3]	K[4]
		Se (95% CI)	Sp (95% CI)	Accuracy		
HP (gm/L)	≥0.09	86.3 (73.7-94.3)	84.3 (71.4-93.0)	85.3	0.71	0.71
SAA (µg/mL)	≥44.43	64.7 (50.1-77.6)	94.1 (83.8-98.8)	79.4	0.59	0.59
IL-1β (pg/mL)	≥125.29	74.5 (60.4-85.7)	94.1 (83.8-98.8)	84.3	0.69	0.69
IFN-γ (pg/mL)	≥77.24	76.5 (62.5-87.2)	90.2 (78.6-96.7)	83.3	0.67	0.67
IL-8 (pg/mL)	≥88.98	96.1 (86.5-99.5)	90.2 (78.6-96.7)	93.1	0.86	0.86
TNF-α (ng/mL)	≥0.92	76.5 (62.5-87.2)	84.3 (71.4-93.0)	80.4	0.61	0.61
PCT (pg/mL)	≥52.36	100 (93.0-100)	96.1 (86.5-99.5)	98.0	0.96	0.96
NPT (ng/mL)	≥4.58	100 (93.0-100)	96.1 (86.5-99.5)	98.0	0.96	0.96

[1] HP = haptoglobin; SAA = serum amyloid A; IL-1β = interleukin 1-beta; IFN-γ = Interferon gamma; IL-8 = interleukin 8; TNF-α = tumor necrosis factor-alpha; PCT = procalcitonin; NPT = neopterin.

[2] Se = sensitivity; Sp = specificity; accuracy = percentage of correctly classified samples.

[3] *J* = Youden index.

[4] κ = Cohen's kappa value.

Fig. 1. Flowchart showing study design, number of bovine respiratory disease infected feedlot calves and number of treated calves.

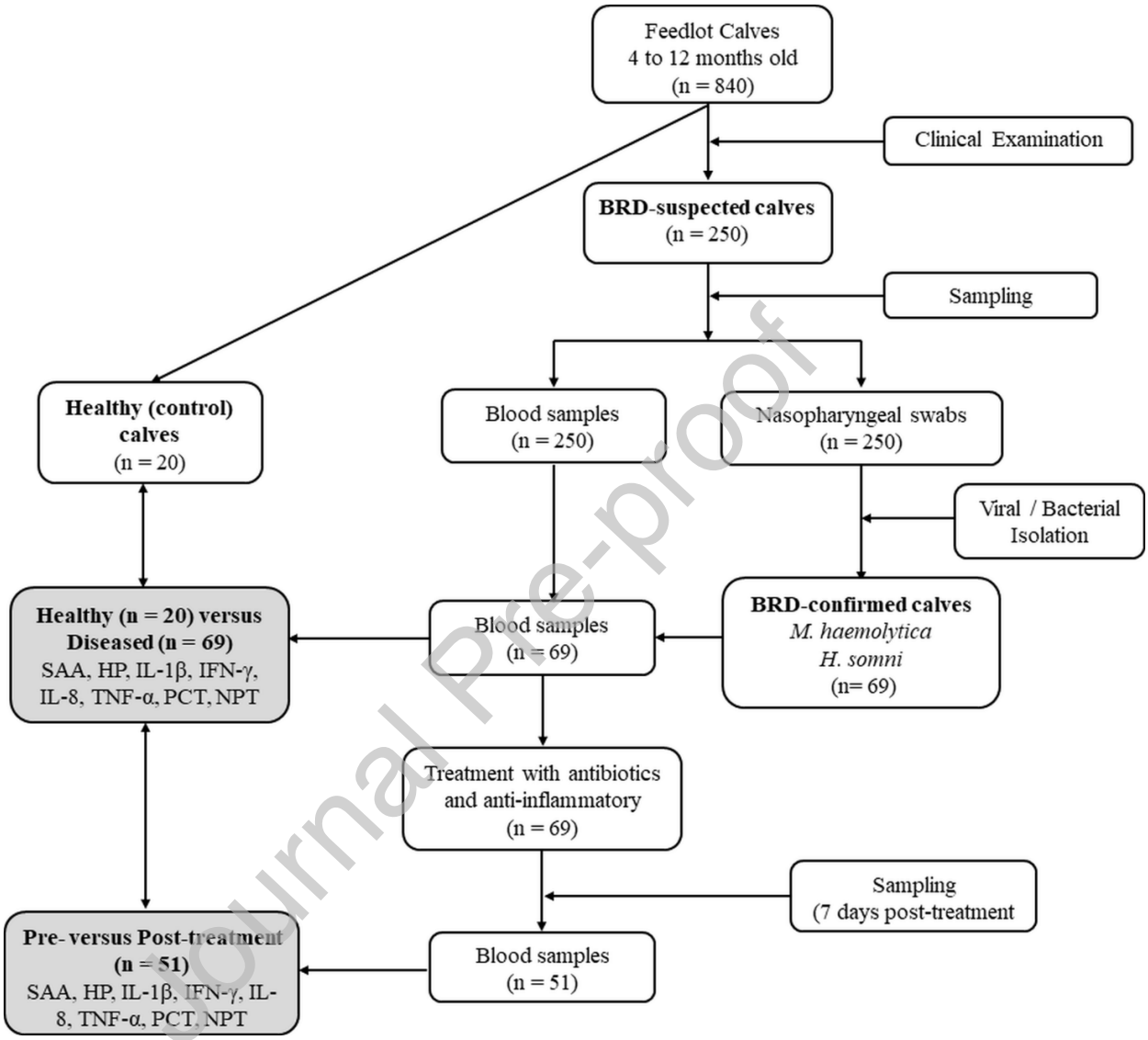


Fig. 2. Box plots illustrating variability of haptoglobin (HP), serum amyloid A (SAA), proinflammatory cytokines (IL-1β, IFN-γ, IL-8, TNF-α), procalcitonin (PCT), and neopterin (NPT) in healthy and bovine respiratory disease infected feedlot calves.

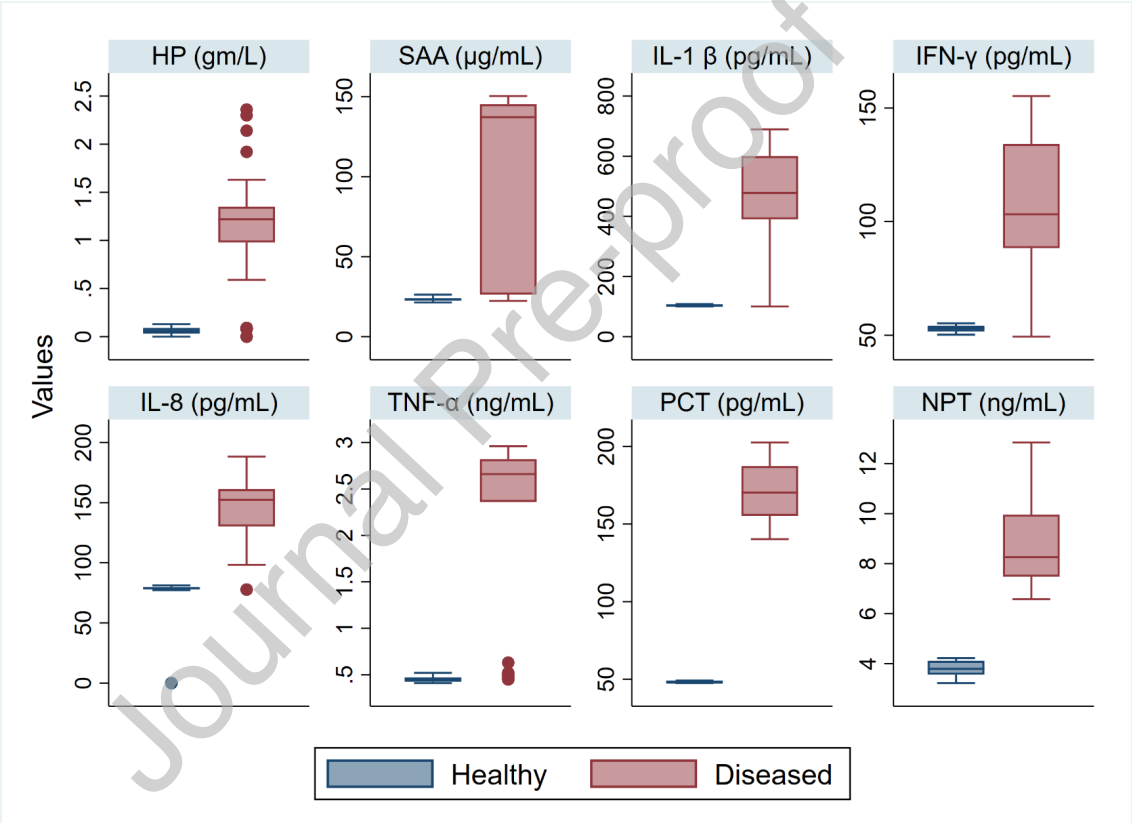


Fig. 3. Box plots illustrating variability of haptoglobin (HP), serum amyloid A (SAA), proinflammatory cytokines (IL-1β, IFN-γ, IL-8, TNF-α), procalcitonin (PCT) and Neopterin (NPT) in bovine respiratory disease infected feedlot calves pre- and 7 days post-treatment.

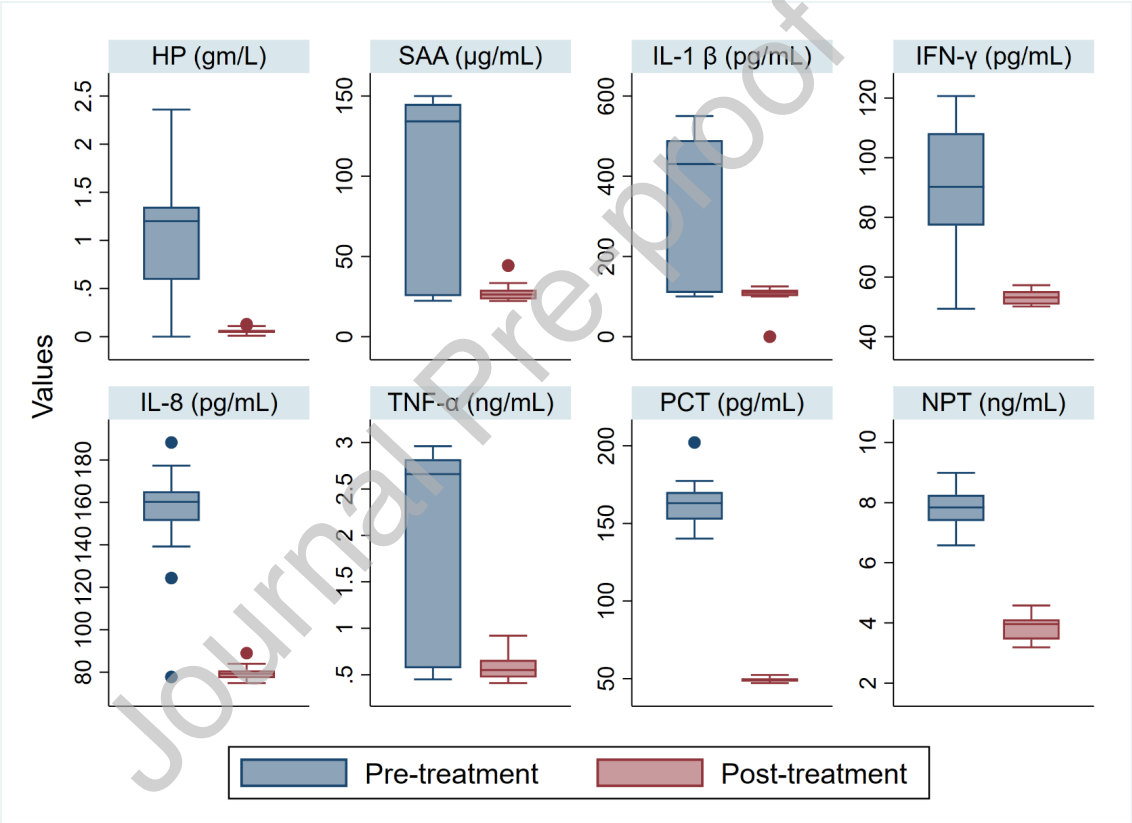


Fig. 4. Receiver operating characteristic (ROC) curve analysis of **(A)** haptoglobin (HP), serum amyloid A (SAA), procalcitonin (PCT) and neopterin (NPT); and **B)** proinflammatory cytokines (IL-1β, IFN- γ, IL-8, and TNF-α) in healthy and bovine respiratory disease infected feedlot calves.

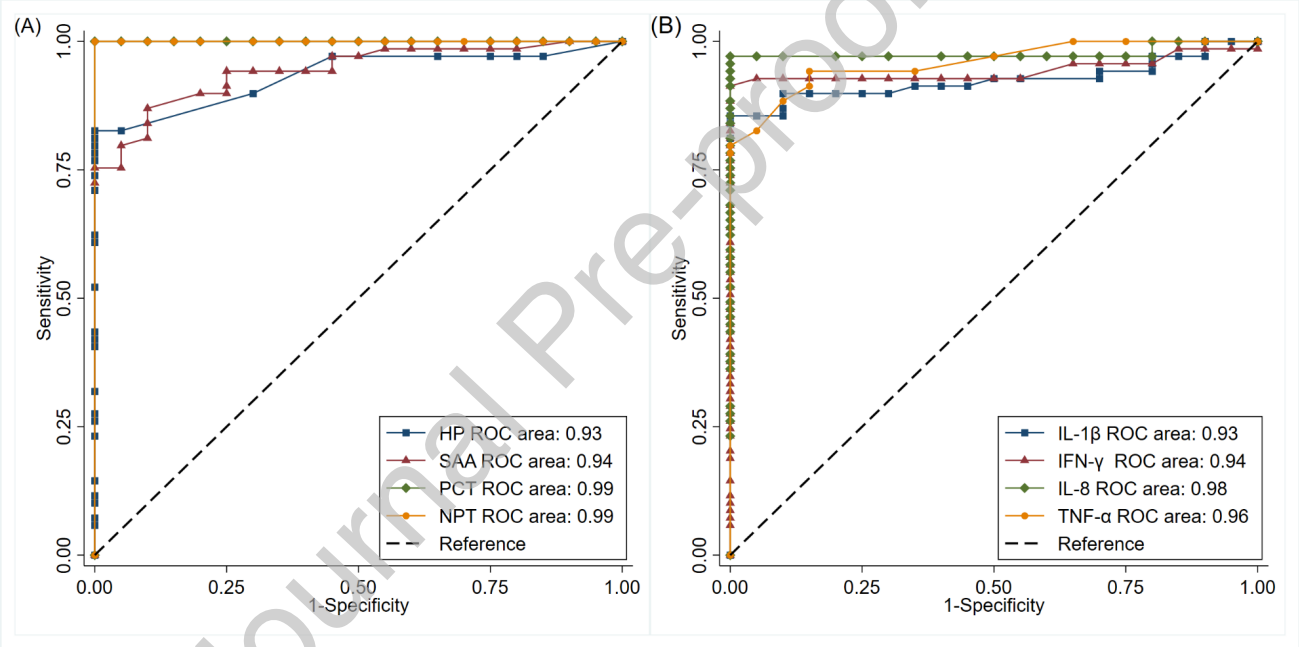
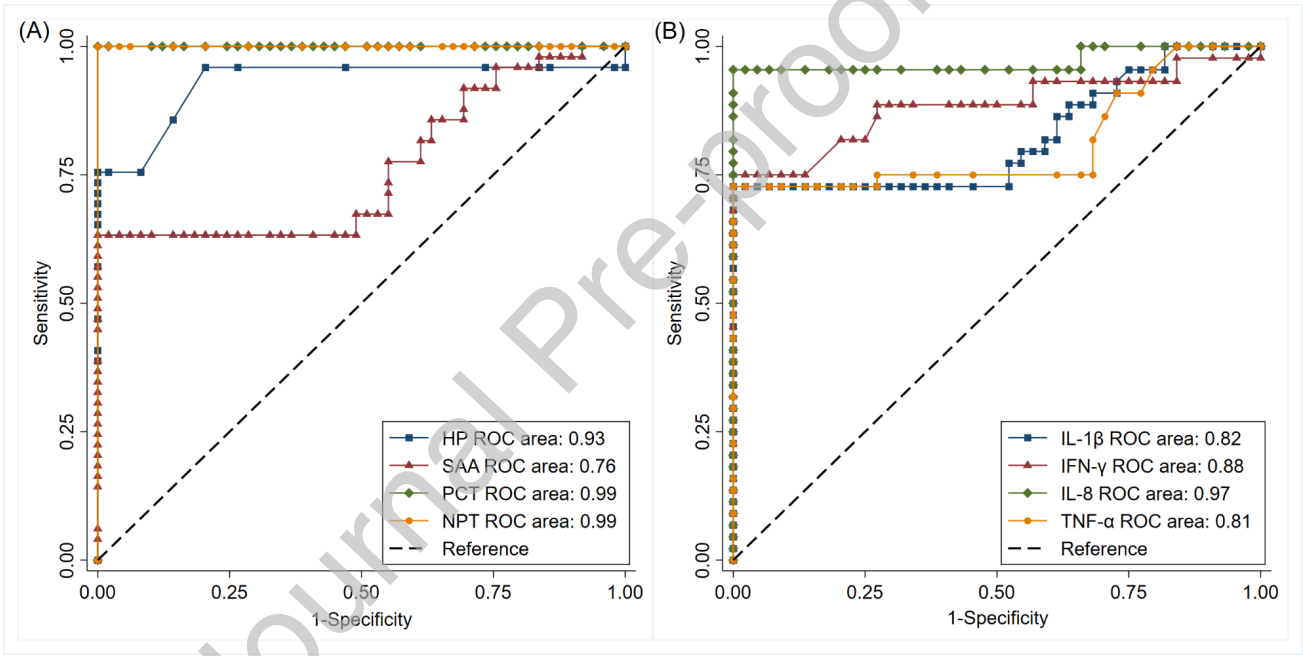


Fig. 5. Receiver operating characteristic (ROC) curve analysis of **(A)** haptoglobin (HP), serum amyloid A (SAA), procalcitonin (PCT) and neopterin (NPT); and **B)** proinflammatory cytokines (IL-1β, IFN- γ, IL-8, and TNF-α) in bovine respiratory disease infected and recovered feedlot calves.



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