Comparative Immunology, Microbiology and Infectious Diseases 67 (2019) 101352

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Acute phase proteins, proinflammatory cytokines and oxidative stress biomarkers in sheep, goats and she-camels with *Coxiella burnetii* infection-induced abortion

Wael El-Deeb[a,b,*], Ibrahim Ghoneim[a,c], Mahmoud Fayez[d,e], Ibrahim Elsohaby[f,g], Abdulrahman Alhaider[a], Magdy ElGioushy[h]

[a] Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa 31982, Al-Hofuf P.O. 400, Saudi Arabia
[b] Department of Veterinary Medicine, Infectious Diseases and Fish Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt
[c] Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[d] Ministry of Agriculture, Al Ahsa Veterinary Diagnostic Laboratory, Saudi Arabia
[e] Veterinary Serum and Vaccine Research Institute, Ministry of Agriculture, Cairo, Egypt
[f] Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt
[g] Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada
[h] Department of Animal Medicine, Faculty of Veterinary Medicine, Aswan University, Aswan, Egypt

ARTICLE INFO

Keywords:
Q fever
Haptoglobin
Serum amyloid A
Cytokines
Abortion
Malondialdehyde

ABSTRACT

Acute phase proteins (APPs) and oxidative stress are helpful markers in diagnosis of several infectious diseases. APPs, proinflammatory cytokines and oxidative stress markers were evaluated for their role in the diagnosis of naturally acquired *Coxiella burnetii* (Q fever) associated with abortion in sheep, goats and she-camels. Blood, aborted materials and vaginal swabs were collected from mixed herds in the Eastern Province of Saudi Arabia. Antioxidant biomarkers showed significant decline in cases of abortion compared to control animals at delivery time. The correlation between disease status and all parameters ranged from moderate to high. The APPs, cytokines and the oxidative stress marker malondialdehyde (MDA) displayed a high degree of distinction between aborted sheep and goat and normal delivered animals (AUC > 0.90). However, only MDA showed a high degree of differentiation (AUC > 0.90) between aborted she-camels and normal delivered controls. In conclusion, results from our study allow us to recommend using APPs, cytokines and oxidative stress markers as an additional tool for diagnosis of naturally occurring *C. burnetii* infection in sheep, goats and she-camels. However, it does not replace standard procedures for detection of *C. burnetii*.

1. Introduction

The Gram-negative intracellular bacterial pathogen *Coxiella burnetii* (*C. burnetii*) is highly infective and persistent [1,2]. These bacteria are known to infect humans and a wide range of farm animals including sheep, cattle, goats [3] and camels [4], causing coxiellosis, or Q fever. In humans, Q fever presents with flu-like symptoms such as headache and myalgia or as atypical pneumonia [5]. *Coxiella burnetii* infection in animals is frequently sub-clinical and associated with such reproductive complaints as late abortion, stillbirth, preterm birth and infertility [6,7]. In addition to being a zoonotic hazard, the economic consequences of Q fever are manifested as a threat to livestock productivity [8].

C. burnetii can only be isolated using embryonated eggs, animal hosts, or mammalian cell culture, which made the diagnosis of Coxiellosis is difficult [9]; however, immune-fluorescence assays and ELISA are practical alternatives for diagnosis of Q fever [10]. When the initial infection occurs, cytokines and chemokines formed by macrophages and monocytes trigger specific immune cells. Inflammatory cytokines such as tumor necrosis factor-alpha can exert harmful effects in the placenta and tend to be present at low concentrations; however, the other regulatory cytokines such as interleukin (IL)-10 is beneficial and tend to predominate [11]. This implies that bacterial challenges that focused on placenta and that produce inflammatory responses may lead to abortion by giving rise to a damaging combination of cytokines that causes damage but does not control the disease [11–13]. Moreover,

* Corresponding author at: Department of Clinical Sciences, College of Veterinary Medicine and Animal Resources, King Faisal University, Al-Ahsa 31982, Al-Hofuf P.O. 400, Saudi Arabia.
E-mail address: weldeeb@kfu.edu.sa (W. El-Deeb).

https://doi.org/10.1016/j.cimid.2019.101352
Received 30 April 2019; Received in revised form 10 September 2019; Accepted 18 September 2019

cytokines are considered as effective motivators for acute phase protein (APP) synthesis [14]. Generally, the acute phase response (APR) is a common immunological reaction of a non-specific pattern appearing before a specific immune response [15–17]. Bacterial, parasitic or viral infections trigger a non-specific APR in various animals [18,19]. Reactive oxygen species (ROS) are natural by-products of normal cellular metabolism, as well as in cellular response to xenobiotics, cytokines, environmental factors and bacterial invasion [20] and cause oxidative damage to the most important cell structures including nucleic acids, carbohydrates, lipids, and proteins [21]. However, the body has numerous means with which to neutralize oxidation by producing antioxidants [22]. The body has antioxidant systems that are frequently valuable in arresting the destructive effects of free radicals [21].

ROS can react with cytokines. The hydroxyl radical is often accountable for oxidative alterations of proteins. Moreover, cytokines can perform antioxidative reactions during acute phase inflammation. ROS cause peroxidative damage to proteins, which lead to modification in amino acid residues and loss of biological activity [23]. Results from previous study has reported that glutathione peroxidase plays a crucial role in regulating proinflammatory responses in the body; failing to regulate these proinflammatory pathways promotes an inflammatory and activated endothelium leading to endothelial dysfunction [24]. The APR can be accountable for changes in concentrations of serum proteins associated with the host response, including antioxidative markers. Inflammatory process could play a significant role in increasing the risk of vascular events associated with increased oxidative stress. The interaction of oxidative stress and inflammatory response has been reported in the inflammation of mammary gland [25].

Erythrocytic glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) are non-enzymatic antioxidants, while ascorbate, vitamin E, urate, glutathione and beta carotene are enzymatic antioxidants [26]. The disturbed equilibrium between antioxidants and free radical levels is referred to as “oxidative stress” [27]. Oxidative stress is one of the early events in disease development [28]. Available literature is lacking with respect to cytokines, APPs and oxidative stress biomarker present in animals infected with *C. burnetii*. Thus, this study aimed to assess the importance of APPs, proinflammatory cytokines and oxidative stress biomarkers as an additional tool for diagnosis of naturally occurring *C. burnetii* infection in sheep, goats and she-camels.

2. Materials and methods

2.1. Study animals

Animals that had aborted (n = 71), including 30 sheep, 20 goats and 21 she-camels from six mixed herds in the Eastern Province of Saudi Arabia, were examined between 2017 and 2018. The herds had a history of abortion due to *C. burnetii* infection. Blood, aborted materials and vaginal swabs were collected from the animals within 2–12 h post abortion. Vaginal discharges and/or portions of placenta were collected and kept in screw-capped bottles. In addition, blood and vaginal swabs were collected within 2–12 h after normal delivery; from 30 clinically healthy animals (10 sheep, 10 goats, and 10 she-camels) that had undergone at least one normal birth. Samples were labeled with the herd name, animal identification number and collection date, and then transported in coolers to the laboratory at King Faisal University. After centrifuging the samples at 1400 × *g* for 15 min at ~20 °C, the collected 3–5 aliquots of serum were kept at −80 °C for further analysis. This study was conducted in accordance with the College of Veterinary Medicine Animal Ethics Committee guidelines, King Faisal University, Saudi Arabia (protocol # 1811013).

2.2. Serological diagnosis of C. burnetii antibodies

Serum samples from the healthy controls and the animals that had aborted were initially screened for the existence of antibodies against abortive diseases predominant in the area. For detection of Brucella antibodies, the Rose Bengale test was used. However, indirect IgG ELISAs were used in antibody testing for *C. burnetii* (CHEKIT Q-Fever Antibody Test Kit, IDEXX Laboratories, ME, USA), *Chlamydophila abortus* (IDEXX Chlamydiosis Total Ab Test, IDEXX Laboratories, ME, USA), and *Toxoplasma gondi* (IDEXX Toxotest Ab Test, IDEXX Laboratories, ME, USA). The ELISAs were performed in accordance with manufacturers’ instructions. Depending on the serological diagnoses, animals were assigned to the healthy, normally delivered, control animal group (samples were seronegative for all tests) or the abortion cases group (samples were only *C. burnetii* seropositive).

2.3. Molecular identification of C. burnetii

Vaginal swabs and abortion materials were inactivated prior DNA extraction. For the extraction of genomic DNA, the Qiagen QIAamp DNA mini-kit (Qiagen SA, Courtaboeuf, France) was used according to the manufacturer's instructions. A 290 bp fragment from the repetitive element IS1111a (Transposase) of the *C. burnetii* genome was amplified with a specific primer (Forward 5`- GTCTTAAGGTGGGCTGCGTG- 3`, Reverse 5`−CCCCGAATCTCATTGATCAGC- 3`) and detected by a specific probe labeled with LightCycler® Red 640 (5`- GTTACTTTTGA CATACGGTTTGACGTGCT- 3`) (TIB MOLBIOL GmbH, Germany). The PCR reagents (Roche Diagnostics, Germany) were used to prepare the reaction mix, and PCR amplification was performed in LightCycler® 2 as described previously [29].

2.4. Acute phase proteins and inflammatory cytokines

Levels of serum haptoglobin (HP) were detected using commercial test kits (Tridelta Development determined Plc.) that measure hemoglobin peroxidase activity, which is in direct proportion to the quantity of HP, whereas, serum amyloid A (SAA) levels were determined via solid sandwich ELISA (Tridelta Development Plc.), following the manufacturer’s instructions. Levels of serum inflammatory cytokine (TNF-α, IL-6 and IL-1β) were determined using ELISA Kits (Cusabio Biotech Co., China) available on the market.

2.5. Oxidative stress markers

In sheep and goats, serum MDA, GSH, SOD and CAT levels were detected via the colorimetric method with commercially purchased test kits (Bio-diagnostic, Egypt). Briefly, when placed in a medium that is acidic at 95 °C for 30 min, MDA reacts with thiobarbituric acid (TBA) and forms a TBA-reactive product that is pink in color with an absorbance that is possible to measure at 534 nm. The GSH level measurement is achieved by reducing 5,5-Dithiobis (2-nitrobenzoic acid) with GSH, which produces a yellow product with an absorbance that is possible to measure at 405 nm. The SOD assay depends on the enzyme’s ability to inhibit the reduction of nitroblue tetrazolium dye, which is mediated by phenazine methosulfate. However, the CAT levels were measured based on the reaction of the catalase enzyme with a specified amount of hydrogen peroxide, as described by Aebi [30].

In she-camels, the MDA, GSH, CAT and SOD levels in the RBC hemolysate were measured using the methods explained previously [31–34].

2.6. Statistical analysis

All statistical analyses were carried out via Stata® Statistics Software (version 15.0; StataCorp, 2017) with results considered significant at $P < 0.05$. Descriptive statistics (mean, standard deviation (SD), median and 25th and 75th percentiles) were calculated for every parameter separately in the healthy and *C. burnetii* infected sheep, goats and she-camels. Because the data were found to significantly deviate from normality, the Wilcoxon-Mann-Whitney test was applied for non-

parametric analysis to evaluate the differences between each parameter in the healthy and *C. burnetii* infected sheep, goats and she-camels. Correlation among different parameters in the sheep, goats and she-camels was assessed by calculating Spearman's rank coefficients of correlation.

A receiver operator characteristic (ROC) curve was applied to each parameter to assess its accuracy in differentiating the healthy sheep, goats and she-camels from those with coxiellosis. The AUC (area under the curve) was calculated and the Youden index (= maximum [sensitivity + specificity-1]) was used to identify the optimal cut-off values for detection of sheep, goats and she-camels with coxiellosis. The diagnostic test characteristics including sensitivity (Se), specificity (Sp) and accuracy of each parameter were calculated. Sensitivity was defined as the proportion of sheep, goats or she-camels with coxiellosis that were correctly identified as such, and Sp was defined as the proportion of healthy sheep, goats or she-camels that were correctly identified as such. Accuracy was defined as the proportion of sheep, goats or she-camels that were correctly classified. Finally, using each parameter, the level of agreement between sheep, goats and she-camels classified as healthy or as having coxiellosis was assessed by Cohen's kappa statistic (κ).

3. Results

Serological examination of the clinically healthy animals (n = 30) detected no antibodies against Brucella, *Chlamydophila abortus*, *Toxoplasma gondii* or *C. burnetii* infections. However, serological examination of the abortion cases (n = 71) showed antibodies against *C. burnetii* infection only. In addition, the target gene IS1111 of *C. burnetii* was detected by real-time polymerase chain reaction (Real-Time PCR) in all abortion cases.

The serum levels of APPs, proinflammatory cytokines and oxidative stress markers in healthy sheep and in sheep with coxiellosis (Q fever) that aborted are presented in Table 1. The concentrations of APPs (HP and SAA), and cytokines (TNF-α, IL-6 and IL-1β) were significantly ($P < 0.001$) higher in the sheep with coxiellosis than in the healthy normally delivered sheep. However, the concentrations of antioxidant markers (GSH, SOD and CAT) were significantly ($P < 0.0001$) lower in the sheep with coxiellosis than in the healthy normally delivered sheep. The level of the oxidative stress marker MDA was significantly ($P < 0.047$) higher in the sheep with coxiellosis than in the healthy controls.

Table 2 shows the concentrations of serum APPs, proinflammatory cytokines and oxidative stress markers in healthy normally delivered goats and in those with coxiellosis. The concentrations of HP, SAA, TNF-α, IL-6, IL-1β and MDA were significantly ($P < 0.001$) higher in the goats with coxiellosis than in the healthy goats. However, the levels of GSH, SOD and CAT were significantly ($P < 0.0001$) lower in the goats with coxiellosis than in the healthy animals.

Table 3 presents the concentrations of serum APPs, proinflammatory cytokines and oxidative stress markers in healthy normally delivered she-camels and in those with coxiellosis. The APPs (HP and SAA) and cytokines (TNF-α and IL-6) were higher in the she-camels with coxiellosis than that in normal delivered she-camels, but did not reach to significance level ($P > 0.05$). A significant increase ($P = 0.0002$) was found in the oxidative stress marker MDA in the she-camels with coxiellosis when compared to the control she-camels. However, there was a significant ($P < 0.001$) decrease in the antioxidant markers (GSH, SOD and CAT) in the she-camels with coxiellosis compared to the healthy normally delivered group.

Spearman's correlation coefficient (r) results among the study parameters in normally delivered sheep, goats and she-camels and in those with coxiellosis that aborted are presented in Table 4. All measured parameters showed a significant ($P < 0.05$) positive correlation with the aborted sheep and goats, but, it did not reach to significant level ($P > 0.05$) with aborted she-camels. Most measured parameters were moderately correlated; however, the correlations were higher in sheep and goats than in she-camels.

Receiver operator characteristic curves were created and the Youden index was calculated to determine the optimum cut-off values for each parameter that indicate the difference between animals with coxiellosis and healthy controls (Fig. 1). Comparison of the AUC of each parameter revealed no significant difference in sheep ($P = 0.35$), goats ($P = 0.13$) or she-camels ($P = 0.18$). The test characteristics (Se, Sp, and accuracy) associated with these optimal thresholds are shown in Table 5 for each parameter and animal group. In addition, the levels of agreement between the number of animals classified as healthy or with coxiellosis by each parameter are presented in Table 5, as assessed using the κ statistic. These results showed that the optimum cut-off values for diagnosis of animals with coxiellosis differed in sheep, goats and she-camels.

4. Discussion

Coxiella burnetii is the pathogenic organism responsible for Q fever, which constitutes a public health problem worldwide. In Saudi Arabia, *C. burnetii* infection is prevalent in camels [35], which, by spreading infection during grazing, are considered the main source in sheep and goats [36]. *Coxiella burnetii* infection in animals is mainly subclinical, with the only prominent sign being abortion, which occurs in up to 80% of cases [6,7]. In the present study, all animals that aborted were serologically diagnosed with *C. burnetii* infection which was corroborated by detection of *C. burnetii* in aborted materials and vaginal swabs using PCR.

The aim of the current study was to address the efficacy of APPs, proinflammatory cytokines and oxidative stress biomarkers as an

Table 1
Descriptive statistics of acute phase proteins (APPs), proinflammatory cytokines and oxidative stress markers in normal delivered and Coxiellosis aborted sheep.

Parameters[a]	Normally delivered sheep (n = 10)					Aborted sheep with Coxiellosis (n = 30)					*P*-value[b]
	Mean	SD	Median	25%	75%	Mean	SD	Median	25%	75%	
HP (g/L)	0.267	0.15	0.30	0.10	0.40	1.87	0.65	1.85	1.57	2.32	< 0.0001
SAA (μg/mL)	10.27	2.84	9.60	7.90	12.40	28.86	7.83	30.26	28.26	33.15	< 0.0001
TNF-α (pg/mL)	7.61	1.30	7.75	6.80	8.40	16.56	3.28	17.26	15.26	18.26	< 0.0001
IL6 (pg/mL)	18.41	3.79	18.77	15.33	20.77	25.63	4.61	27.14	24.33	28.36	0.0004
IL1-β (pg/mL)	16.65	2.27	15.71	15.22	18.55	22.71	4.60	23.30	20.36	25.26	0.0008
MDA (nmol/mL)	6.51	0.86	6.25	5.80	7.30	8.67	3.04	7.76	6.26	11.25	0.0473
GSH (mg/dL)	1.25	0.23	1.25	1.15	1.36	0.58	0.34	0.48	0.39	0.56	0.0002
SOD (U/mL)	153.30	7.32	153.99	149.66	158.88	110.21	19.53	109.76	98.26	114.36	0.0001
CAT (U/L)	299.13	49.59	329.66	233.15	335.22	180.78	9.65	180.81	174.26	187.26	< 0.0001

[a] HP, haptoglobin; SAA, serum amyloid A; TNF-α, tumor necrosis factor-alpha; IL6, interleukin 6; IL1-β, interleukin 1- alpha; MDA, malondialdehyde; GSH, erythrocytic glutathione; SOD, superoxide dismutase; CAT, catalase.
[b] *P* value: resulting from non-parametric Wilcoxon-Mann-Whitney test.

Table 2
Descriptive statistics of acute phase proteins (APPs), proinflammatory cytokines and oxidative stress markers in normal delivered and Coxiellosis aborted goats.

Parameters[a]	Normally delivered goats (n = 10)					Aborted goats with Coxiellosis (n = 20)					*P*-value[b]
	Mean	SD	Median	25%	75%	Mean	SD	Median	25%	75%	
HP (g/L)	0.27	0.15	0.30	0.30	0.40	1.21	0.68	1.1	0.75	1.65	0.0003
SAA (μg/mL)	7.47	1.90	7.85	6.60	8.70	17.94	7.33	18.74	13.81	20.81	0.0003
TNF-α (pg/mL)	9.47	2.83	9.63	6.87	10.40	19.39	4.90	20.36	19.35	22.13	0.0004
IL6 (pg/mL)	16.05	1.85	16.21	14.33	17.55	28.88	14.11	28.30	22.61	31.3	0.0013
IL1-β (pg/mL)	15.15	2.41	15.82	13.77	16.55	27.47	6.41	28.26	23.77	32.07	0.0002
MDA (nmol/mL)	6.61	0.67	6.40	6.30	7.20	10.53	1.08	10.33	9.79	11.26	< 0.0001
GSH (mg/dL)	1.01	0.14	0.99	0.90	1.14	0.63	0.11	0.59	0.55	0.71	< 0.0001
SOD (U/mL)	154.23	4.15	152.55	152.2	155.55	109.46	7.98	110.85	100.36	114.36	< 0.0001
CAT (U/L)	309.54	23.09	307.66	288.78	332.65	179.07	14.76	183.76	168.26	190.26	< 0.0001

[a] HP, haptoglobin; SAA, serum amyloid A; TNF-α, tumor necrosis factor-alpha; IL6, interleukin 6; IL1-β, interleukin 1- alpha; MDA, malondialdehyde; GSH, erythrocytic glutathione; SOD, superoxide dismutase; CAT, catalase.
[b] *P* value: resulting from non-parametric Wilcoxon-Mann-Whitney test.

Table 3
Descriptive statistics of acute phase proteins (APPs), proinflammatory cytokines and oxidative stress markers in normal delivered and Coxiellosis aborted she-camels.

Parameters[a]	Normally delivered she-camels (n = 10)					Aborted she-camels with Coxiellosis (n = 21)					*P*-value[b]
	Mean	SD	Median	25%	75%	Mean	SD	Median	25%	75%	
HP (g/L)	0.38	0.07	0.37	0.33	0.45	1.10	0.80	1.29	0.32	1.71	0.1502
SAA (μg/mL)	10.01	0.70	9.80	9.50	10.50	14.49	4.84	16.10	9.50	18.10	0.1388
TNF-α (pg/mL)	16.12	1.06	15.87	15.33	16.88	24.54	9.49	25.60	15.20	33.80	0.2048
IL6 (pg/mL)	14.52	0.76	14.63	13.75	14.88	16.43	3.96	16.90	12.60	20.10	0.6725
IL1-β (pg/mL)	23.29	1.62	22.61	21.99	24.77	26.37	5.91	22.50	21.80	32.60	0.7996
MDA (nmol/mL)	12.13	1.06	12.20	11.44	12.70	21.48	3.70	22.45	22.36	23.12	0.0002
GSH (mg/dL)	6.54	0.81	6.55	5.70	7.30	4.64	2.06	3.90	3.80	4.20	0.0003
SOD (U/mL)	4.79	0.36	4.70	4.60	5.20	4.23	1.55	3.80	3.60	4.10	0.0009
CAT (U/L)	15.58	0.87	15.66	14.77	16.27	12.00	2.14	12.30	9.80	13.60	0.0001

[a] HP, haptoglobin; SAA, serum amyloid A; TNF-α, tumor necrosis factor-alpha; IL6, interleukin 6; IL1-β, interleukin 1- alpha; MDA, malondialdehyde; GSH, erythrocytic glutathione; SOD, superoxide dismutase; CAT, catalase.
[b] *P* value: resulting from non-parametric Wilcoxon-Mann-Whitney test.

Table 4
Correlation matrix among acute phase proteins (APPs) and proinflammatory cytokines in normal delivered and Coxiellosis aborted sheep, goats and she-camels.

Parameters	Normal / Aborted	HP	SAA	TNF-α	IL6	IL1-β
Sheep (n = 45)						
HP	0.65[c]	1.00				
SAA	0.65[c]	0.60[c]	1.00			
TNF-α	0.67[c]	0.41[b]	0.59[c]	1.00		
IL6	0.57[c]	0.29[a]	0.35[a]	0.38[b]	1.00	
IL1-β	0.54[c]	0.21	0.34[a]	0.42[b]	0.25	1.00
Goats (n = 30)						
HP	0.67[c]	1.00				
SAA	0.67[c]	0.44[c]	1.00			
TNF-α	0.66[c]	0.56[c]	0.57[c]	1.00		
IL6	0.60[c]	0.29	0.37[a]	0.51[b]	1.00	
IL1-β	0.69[c]	0.51[b]	0.53[b]	0.41[a]	0.41[a]	1.00
She-camels (n = 31)						
HP	0.26	1.00				
SAA	0.27	0.76[c]	1.00			
TNF-α	0.23	0.44[b]	0.61[c]	1.00		
IL6	0.17	0.77[c]	0.81[c]	0.58[c]	1.00	
IL1-β	0.14	0.48[b]	0.32[a]	0.17	0.52[b]	1.00

[a] Significant correlations at $P < 0.05$.
[b] Significant correlations at $P < 0.01$.
[c] Significant correlations at $P < 0.001$.

additional tool for diagnosis of *C. burnetii* infection-induced cases of abortion. In ruminants, the dominant APPs are HP and SAA, which are synthesized in the hepatocytes [37]. These proteins vary in concentration during infections, inflammation, surgical trauma and stress [15]. The current findings revealed considerably higher HP and SAA levels in *C. burnetii* infected sheep and goats. It is likely that the significantly elevated proinflammatory cytokines recorded in this study mediated the significant increase of HP and SAA [38]. The HP binds the free hemoglobin, thus preventing the pathogenic bacteria from accessing the iron requisite for their development [39]. On the other hand, SAA binds to bacteria (Gram-negative) causing opsonization of the target microorganism [40]. It was supposed that the species variations in the levels of HP and SAA reported in the present study stemmed from differences in production and in the frequency of diffusion of these APPs from the blood of the infected animals [41,42]. Moreover, the concentrations of HP demonstrated the seriousness of the disease and resultant fundamental tissue damage [37]. Similar findings have been reported in aborted goats with Border disease [43], aborted cows with brucellosis [44] and aborted mares due to infection with *Streptococcus zooepidemicus* [45].

The evident increase of serum cytokines reported in the infected animals in this study indicated that the monocytes and macrophages were stimulated to secret proinflammatory cytokines in response to a stressful situation (abortion) [46]. Cytokines are involved in the elimination process of *C. burnetii* in infected animals [47,48]. The species variations recorded in the concentration of cytokines can possibly be attributed to a variation within cytokine genes [49]. The substantial increase in proinflammatory cytokines in the sheep and goat abortion cases in the present study was comparable to that described in ovine chlamydial abortion [50].

The elevated cytokines in the serum of animals that had aborted mediated the effect of the positive APPs (HP and SAA). They were also increasingly expressed in the sheep, goat and she-camel abortion cases to trigger differentiation of the T-helper cells that create a link between

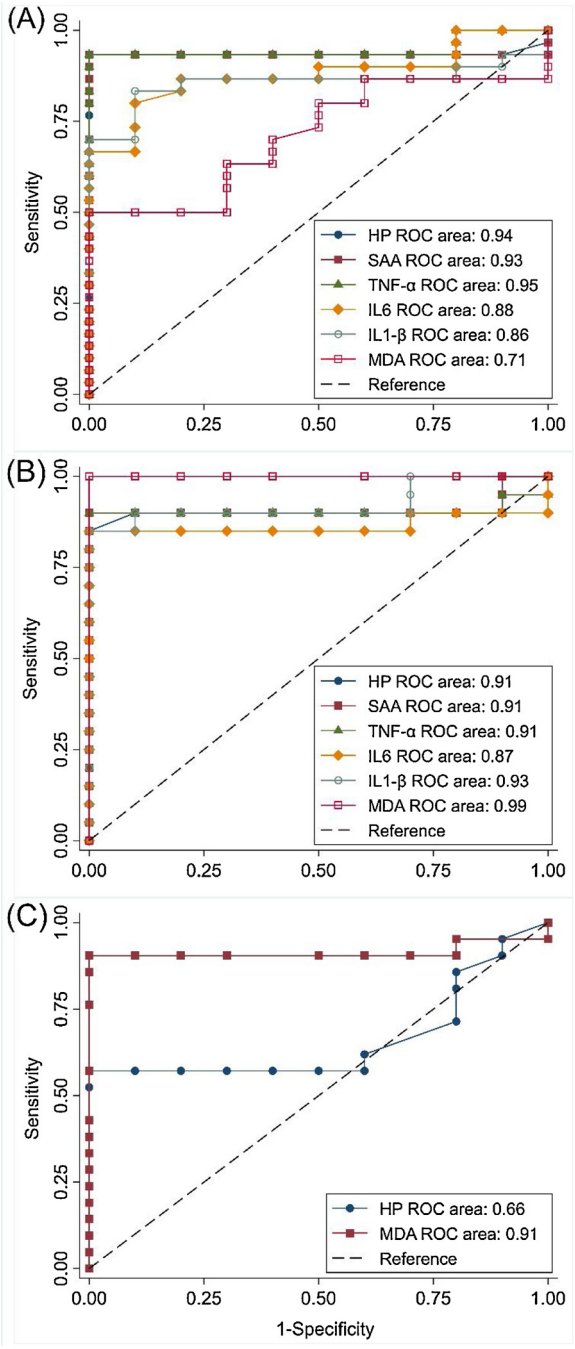

Fig. 1. Receiver operating characteristic (ROC) curve analyses of acute phase proteins (APP), proinflammatory cytokines and oxidative stress markers in normally delivered (**A**) sheep, (**B**) goats and (**C**) she-camels and those aborted with Coxiellosis.

inborn resistance and an adaptive immune response [51].

The variation in levels of inflammatory response against infection with *C. burnetii* between small ruminants (sheep and goat) and she-camels in this study may be attributed to the special physiological properties of camels and its different genomic structure [52]. Moreover, the dose of infection and rate of damaged cells may also a major factor affecting the inflammatory response in animals under investigation [53].

Oxidative stress has been recorded in various infectious and non-infectious syndromes of domestic animals [19,54]. The oxidative stress can be determined by measuring the oxidant and antioxidant activity [55]. Lipids are very predisposed to the oxidation process and lipid peroxidation products were measured as potential biomarkers for that state of oxidative stress. The final product of fatty acids peroxidation in the cells is MDA. Increased levels of free radicals in the circulation cause over-synthesis of MDA [16,56,57]. The substantial increase of MDA levels in infected sheep, goats and she-camels recorded in this study indicated excessive lipid peroxidation [16,56]. Lipid peroxidation is created by the frequent discharge of oxygen-free radicals due to bacterial infection or reduced antioxidants levels [22,58].

The exhaustion of SOD, GSH and CAT and their protection against oxidative stress in the cells may explain the low concentration of these enzymes reported in the infected animals [59]. In the event that antioxidants proceeding from within the cell (SOD, GSH and CAT) do not eliminate these ruinous radicals, the oxidation rate will surpass the antioxidation rate, finally resulting in oxidative stress [58]. These actions were accurately perceived in this investigation as the serum of animals with *C. burnetii* showed substantially higher levels of MDA and lower concentrations of SOD, GSH, and CAT activity when compared with healthy normally delivered controls. In this study it was noticeable that depletion of GSH, SOD and CAT in the serum of sheep, goats and she-camels with *C. burnetii* infection was credited with the reduced ability of the antioxidant enzyme system to neutralize the oxidative stress state. Results from previous study showed significant increase in MDA levels and a significant decrease in GSH and SOD in aborted goats with border disease than control one [43].

The ability of APPs, proinflammatory cytokines and MDA to discriminate between animals with *C. burnetii* and healthy sheep, goats and she-camels was assessed using ROC analysis. In this context, all examined APPs (HP and SAA) displayed a high level of distinction between the sheep and goats that had aborted and the healthy ones (AUC $>$ 0.90), in accordance with the guidelines described by Swets [60]. Nonetheless, only HP displayed a moderate degree of distinction between she-camels that had aborted and healthy normally delivered ones (AUC $>$ 0.70). Furthermore, a high degree of differentiation of MDA was revealed between sheep, goats and she-camels that had aborted and healthy ones (AUC $>$ 0.90). The capacity of HP and SAA to distinguish between healthy normally delivered animals and infected cases might be attributed to the huge contrast in the serum levels between the controls and the coxiellosis cases. Similarly, proinflammatory cytokines revealed a comparable high diagnostic performance (AUC $>$ 0.85) in sheep and goats that had aborted. These findings also specified the high level of diagnostic accuracy exhibited by the cytokines observed in the study. Nevertheless, APPs may be considered as superior biological markers for detecting inflammation, in view of the extremely short half-life of cytokines in the circulation.

Selection of the optimal threshold for achieving the highest Se, Sp and correct classification (accuracy) for each parameter was based on the Youden index. As HP, SAA, TNF-α, IL-6, IL-β, and MDA exhibited the maximum Se and Sp, they can be considered as suitable indicators for acute phase response and oxidative stress profiles in *C. burnetii* infected sheep and goats. As HP and MDA demonstrated a better degree of Se and Sp in recognizing *C. burnetii* infected she-camels, this suggests that they can be considered as sensitive markers for the disease in this species.

5. Conclusions

Our results indicated that abortion caused by *C. burnetii* in sheep and goats was linked to a significant rise in APPs and proinflammatory cytokines, with SAA and TNF-α representing the highest increases. However, abortion caused by *C. burnetii* in sheep, goats and she-camel was linked to significant decline in oxidative stress markers. The correlation of disease status with APPs and cytokines was moderate to high. Furthermore, results from the present study suggest that APPs, cytokines and oxidative stress markers can be used as an additional useful tool for diagnosis of natural occurrence of Q fever in cases of abortion among sheep, goats and she-camels. But it does not replace

Table 5
Test characteristics of acute phase proteins (APP), proinflammatory cytokines and oxidative stress markers in normal delivered and Coxiellosis aborted sheep, goats and she-camels.

Parameters	Threshold	Diagnostic characteristics (%)[a]			J[b]	κ[c]	Coxiellosis -/+	Test -/+
		Se (95% CI)	Sp (95% CI)	Accuracy				
Sheep (n = 40)								
HP (g/L)	≥1.5	93.3 (77.9–99.2)	100 (69.2–100)	95.0	0.93	0.88	10/30	12/28
SAA (μg/mL)	≥22.23	90.0 (73.5–97.9)	100 (69.2–100)	92.5	0.90	0.82	10/30	13/27
TNF-α (pg/mL)	≥14.22	93.3 (77.9–99.2)	100 (69.2–100)	92.5	0.93	0.88	10/30	13/27
IL6 (pg/mL)	≥25.12	66.7 (47.2–82.7)	100 (69.2–100)	75.0	0.67	0.50	10/30	20/20
IL1-β (pg/mL)	≥21.36	70.0 (50.6–85.3)	100 (69.2–100)	77.5	0.70	0.54	10/30	10/21
MDA (nmol/mL)	≥6.26	76.7 (57.7–90.1)	50.0 (18.7–81.3)	70.0	0.27	0.25	10/30	12/28
Goats (n = 30)								
HP (g/L)	≥0.5	90.0 (68.3-98.8)	90.0 (55.5-99.7)	90.0	0.80	0.78	10/20	11/19
SAA (μg/mL)	≥10.69	85.0 (62.1-96.8)	100 (69.2-100)	90.0	0.85	0.79	10/20	13/17
TNF-α (pg/mL)	≥18.15	85.0 (62.1-96.8)	100 (69.2-100)	90.0	0.85	0.79	10/20	13/17
IL6 (pg/mL)	≥18.75	85.0 (62.1-96.8)	90.0 (55.5-99.7)	90.0	0.80	0.78	10/20	12/18
IL1-β (pg/mL)	≥20.26	85.0 (62.1-96.8)	100 (69.2-100)	90.0	0.85	0.79	10/20	13/17
MDA (nmol/mL)	≥7.8	100 (83.2-100)	90.0 (55.5-99.7)	96.7	0.90	0.92	10/20	9/21
She-camels (n = 31)								
HP (g/L)	≥0.47	57.1 (34.0-78.2)	100 (69.2-100)	71.0	0.57	0.46	10/21	12/19
MDA (nmol/mL)	≥20.36	90.5 (69.1-98.8)	100 (69.2-100)	93.6	0.91	0.86	10/21	12/19

[a] Se = sensitivity; Sp = specificity; accuracy = percentage of correctly classified samples.
[b] J = Youden index.
[c] κ = Cohen's kappa value.

standard procedures for detection of *C. burnetii*.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgement

The authors express their gratitude to the King Faisal University, Deanship of Scientific Research for their financial support of this study (Project no: 1811013).

References

[1] R.A. Heinzen, T. Hackstadt, J.E. Samuel, Developmental biology of Coxiella burnetii, Trends Microbiol. 7 (1999) 149–154.
[2] T.F. McCaul, J.C. Williams, Developmental cycle of *Coxiella burnetii*: structure and morphogenesis of vegetative and sporogenic differentiations, J. Bacteriol. 147 (1981) 1063–1076.
[3] E. Angelakis, D. Raoult, Q fever, Vet. Microbiol. 140 (2010) 297–309.
[4] H.J. Pirouz, G. Mohammadi, J. Mehrzad, M. Azizzadeh, M.H.N. Shirazi, Seroepidemiology of Q fever in one-humped camel population in northeast Iran, Trop. Anim. Health Prod. 47 (2015) 1293–1298.
[5] H. Honarmand, Q fever: an old but still a poorly understood disease, Interdiscip. Perspect. Infect. Dis. 2012 (2012) 1–8.
[6] G.H. Lang, Serosurvey of *Coxiella burnetii* infection in dairy goat herds in Ontario. A comparison of two methods of enzyme-linked immunosorbent assay, Can. J. Vet. Res. 52 (1988) 37.
[7] J.S. Agerholm, *Coxiella burnetii* associated reproductive disorders in domestic animals - a critical review, Acta Vet. Scand. 55 (2013) 13.
[8] M. Van Asseldonk, D.M. Bontje, J.A. Backer, H. van Roermund, R. Bergevoet, Economic aspects of Q fever control in dairy goats, Prev. Vet. Med. 121 (2015) 115–122.
[9] K. Boden, K. Wolf, B. Hermann, D. Frangoulidis, First isolation of *Coxiella burnetii* from clinical material by cell-free medium (ACCM2), Eur. J. Clin. Microbiol. Infect. Dis. 34 (2015) 1017–1022.
[10] E.J. Van Schaik, C. Chen, K. Mertens, M.M. Weber, J.E. Samuel, Molecular pathogenesis of the obligate intracellular bacterium Coxiella burnetii, Nat. Rev. Microbiol. 11 (2013) 561.
[11] G. Entrican, Immune regulation during pregnancy and host–pathogen interactions in infectious abortion, J. Comp. Pathol. 126 (2002) 79–94.
[12] B. Henderson, S. Poole, M. Wilson, Microbial/host interactions in health and disease: who controls the cytokine network? Immunopharmacology 35 (1996) 1–21.
[13] M.P. Murtaugh, M.J. Baarsch, Y. Zhou, R.W. Scamurra, G. Lin, Inflammatory cytokines in animal health and disease, Vet. Immunol. Immunopathol. 54 (1996) 45–55.
[14] G.W. Duff, Cytokines and acute phase proteins in rheumatoid arthritis, Scand. J. Rheumatol. 23 (1994) 9–19.
[15] H.H. Petersen, J.P. Nielsen, P.M.H. Heegaard, Application of acute phase protein measurements in veterinary clinical chemistry, Vet. Res. 35 (2004) 163–187.
[16] W.M. El-Deeb, M. Tharwat, Lipoproteins profile, acute phase proteins, proinflammatory cytokines and oxidative stress biomarkers in sheep with pneumonic pasteurellosis, Comp. Clin. Path. 24 (2015) 581–588.
[17] W.M. El-Deeb, A.M. Elmoslemany, The diagnostic accuracy of acute phase proteins and proinflammatory cytokines in sheep with pneumonic pasteurellosis, PeerJ. 4 (2016) e2161.
[18] P.D. Eckersall, R. Bell, Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine, Vet. J. 185 (2010) 23–30.
[19] W.M. El-Deeb, S. Buczinski, The diagnostic and prognostic importance of oxidative stress biomarkers and acute phase proteins in urinary tract infection (UTI) in camels, PeerJ. 3 (2015) e1363.
[20] A. Bhattacharyya, R. Chattopadhyay, S. Mitra, S.E. Crowe, Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases, Physiol. Rev. 94 (2014) 329–354.
[21] E. Birben, U.M. Sahiner, C. Sackesen, S. Erzurum, O. Kalayci, Oxidative stress and antioxidant defense, World Allergy Organ. J. 5 (2012) 9.
[22] R.K. Gupta, A.K. Patel, N. Shah, A. Chaudhary, U. Jha, U.C. Yadav, et al., Oxidative stress and antioxidants in disease and cancer, Asian Pac. J. Cancer Prev. 15 (2014) 4405–4409.
[23] M. Kankofer, Protein peroxidation processes in bovine retained and not-retained placenta, J. Vet. Med. Ser. A 48 (2001) 207–212.
[24] A. Sharma, D. Yuen, O. Huet, R. Pickering, N. Stefanovic, P. Bernatchez, et al., Lack of glutathione peroxidase-1 facilitates a pro-inflammatory and activated vascular endothelium, Vasc. Pharmacol. 79 (2016) 32–42.
[25] M. Kleczkowski, W. Kluciński, M. Czerski, E. Kudyba, Association between acute phase response, oxidative status and mastitis in cows, Vet. Stanica 48 (3) (2017) 177–186.
[26] J.K. Miller, E. Brzezinska-Slebodzinska, F.C. Madsen, Oxidative stress, antioxidants, and animal function, J. Dairy Sci. 76 (1993) 2812–2823.
[27] M. Valko, D. Leibfritz, J. Moncol, M.T. Cronin, M. Mazur, J. Telser, Free radicals and antioxidants in normal physiological functions and human disease, Int. J. Biochem. Cell Biol. 39 (2007) 44–84.
[28] J. Lykkesfeldt, O. Svendsen, Oxidants and antioxidants in disease: Oxidative stress in farm animals, Vet. J. 173 (2007) 502–511.
[29] M. Stemmler, H. Meyer, Rapid and specific detection of *coxiella burnetii* by LightCycler PCR, Anonymous Rapid Cycle Real-Time PCR—Methods and Applications, Springer, 2002, pp. 149–154.
[30] H. Aebi, Catalase in vitro, Methods Enzymol. 105 (1984) 121–126.
[31] Z.A. Placer, L.L. Cushman, B.C. Johnson, Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems, Anal. Biochem. 16 (1966) 359–364.
[32] E. Beutler, Improved method for the determination of blood glutathione, J. Lab. Clin. Med. 61 (1963) 882–888.
[33] H.P. Misra, I. Fridovich, The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase, J. Biol. Chem. 247 (1972) 3170–3175.
[34] R.F. Beers, I.W. Sizer, A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, J. Biol. Chem. 195 (1952) 133–140.
[35] M.F. Hussein, M.A. Alshaikh, R.S. Al-Jumaah, A. GarelNabi, I. Al-Khalifa, O.B. Mohammed, The Arabian camel (*camelus dromedarius*) as a major reservoir of Q fever in Saudi Arabia, Comp. Clin. Path. 24 (2015) 887–892.
[36] M.M. Fassi-Fehri, Diseases of camels, Rev. Sci. Tech. Off. Int. Epiz. 6 (1987) 337–354.

[37] P.M. Heegaard, D.L. Godson, M.J. Toussaint, K. Tjørnehøj, L.E. Larsen, B. Viuff, et al., The acute phase response of haptoglobin and serum amyloid A (SAA) in cattle undergoing experimental infection with bovine respiratory syncytial virus, Vet. Immunol. Immunopathol. 77 (2000) 151–159.
[38] S. Jain, V. Gautam, S. Naseem, Acute-phase proteins: As diagnostic tool, J. Pharm. Bioallied Sci. 3 (2011) 118.
[39] J.W. Eaton, P. Brandt, J.R. Mahoney, J.T. Lee, Haptoglobin: A natural bacteriostat, Science 215 (1982) 691–693.
[40] R. Hari-Dass, C. Shah, D.J. Meyer, J.G. Raynes, Serum amyloid A protein binds to outer membrane protein A of gram-negative bacteria, J. Biol. Chem. 280 (2005) 18562–18567.
[41] P.D. Eckersall, Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals, Rev. Med. Vet. 151 (2000) 577–584.
[42] J.J. Kaneko, Serum proteins and the dysproteinemias, Anonymous Clinical Biochemistry of Domestic Animals, Elsevier, 1997, pp. 117–138.
[43] E. Balikci, A. Yildiz, F. Gurdogan, Selected acute phase proteins, oxidative stress biomarkers, and antioxidants in aborting and non-aborting goats infected with border disease virus, Bull. Vet. Inst. Pulawy 57 (2013) 371–374.
[44] H. Sharifiyazdia, S. Nazifi, K. Nikseresht, R. Shahriari, Evaluation of serum amyloid A and haptoglobin in dairy cows naturally infected with brucellosis, J. Bacteriol. Parasitol. 3 (2012) 157–161.
[45] M.A. Coutinho da Silva, I.F. Canisso, M.L. MacPherson, A. Johnson, T.J. Divers, Serum amyloid A concentration in healthy periparturient mares and mares with ascending placentitis, Equine Vet. J. 45 (2013) 619–624.
[46] M.T. Peraçoli, C.S. Kurokawa, S.A. Calvi, R.P. Mendes, P.C. Pereira, S.A. Marques, et al., Production of pro-and anti-inflammatory cytokines by monocytes from patients with paracoccidioidomycosis, Microb. Infect. 5 (2003) 413–418.
[47] J. Turco, H.A. Thompson, H.H. Winkler, Interferon-gamma inhibits growth of *Coxiella burnetii* in mouse fibroblasts, Infect. Immun. 45 (1984) 781–783.
[48] J. Dellacasagrande, C. Capo, D. Raoult, J. Mege, IFN-γ-mediated control of *Coxiella burnetii* survival in monocytes: the role of cell apoptosis and TNF, J. Immunol. 162 (1999) 2259–2265.
[49] A.K. Turner, M. Begon, J.A. Jackson, J.E. Bradley, S. Paterson, Genetic diversity in cytokines associated with immune variation and resistance to multiple pathogens in a natural rodent population, PLoS Genet. 7 (2011) e1002343.
[50] D. Buxton, I.E. Anderson, D. Longbottom, M. Livingstone, S. Wattegedera, G. Entrican. Ovine chlamydial abortion: characterization of the inflammatory immune response in placental tissues, J. Comp. Pathol. 127 (2002) 133–141.
[51] G. Trinchieri, Interleukin-12 and the regulation of innate resistance and adaptive immunity, Nat. Rev. Immunol. 3 (2003) 133.
[52] L. Andersson, M. Georges, Domestic-animal genomics: deciphering the genetics of complex traits, Nat. Rev. Genet. 5 (2004) 202–212.
[53] L. Chen, H. Deng, H. Cui, J. Fang, Z. Zuo, J. Deng, et al., Inflammatory responses and inflammation-associated diseases in organs, Oncotarget 9 (2018) 7204–7218.
[54] D. Rodríguez de Sotillo, A.M. Velly, M. Hadley, J.R. Fricton, Evidence of oxidative stress in temporomandibular disorders: a pilot study, J. Oral Rehabil. 38 (2011) 722–728.
[55] P. Celi, Biomarkers of oxidative stress in ruminant medicine, Immunopharmacol. Immunotoxicol. 33 (2011) 233–240.
[56] A. Ayala, M.F. Muñoz, S. Argüelles, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, Oxid. Med. Cell. Longev. 2014 (2014).
[57] C. Castillo, J. Hernandez, A. Bravo, M. Lopez-Alonso, V. Pereira, J.L. Benedito, Oxidative status during late pregnancy and early lactation in dairy cows, Vet. J. 169 (2005) 286–292.
[58] H. Sies, Oxidative stress: oxidants and antioxidants, Exp. Physiol. 82 (1997) 291–295.
[59] B. Halliwell, Antioxidants in human health and disease, Annu. Rev. Nutr. 16 (1996) 33–50.
[60] J.A. Swets, Measuring the accuracy of diagnostic systems, Science 240 (1988) 1285–1293.