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RESEARCH ARTICLE

Antioxidant Status and Biochemical Alterations in *Chlamydia abortus and Coxiella burnetii* Infected Small Ruminants

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ABSTRACT

Chlamydia abortus and Coxiella burnetii are gram-negative abortifacient bacteria that cause abortion in livestock animals, thus, leading to severe economic losses to farmers. This study aimed to assess oxidative stress and serum-biochemical changes due to *C. abortus* and *C. burnetii* infection in small ruminants. A total of 168 serum samples from sheep (n=84) and goats (n=84) were tested for the presence of antigen-specific antibodies against *C. abortus* and *C. burnetii* using indirect ELISA. The serum samples of seropositive and healthy animals were tested to determine the concentration of catalase and malondialdehyde (MDA) using standard laboratory procedures. The concentration of total protein (TP) and albumin in serum was determined using commercially available kits. Results of ELISA showed that the number of positive samples of *C. abortus* was 2.4% in sheep and 7.1% in goats, whereas that of *C. burnetii* was 13.1% in sheep and 25% in goats. The catalase concentration in seropositive animals (1.39±0.27 kU/l) was significantly lower ($P<0.05$) whereas that of MDA (2.90±0.82 µM/l) was higher than in healthy animals i.e., 2.65±0.55 and 1.25±0.85, respectively. The total protein and albumin concentration of *C. abortus* affected animals was 6.4±0.4 g/dL and 3.1±0.4 g/dL, respectively and found significantly lower ($P<0.05$) than that of healthy ones (7.4±0.3 g/dL and 3.7±0.3 g/dL). Statistical analysis revealed that species and flock type played a significant part ($P<0.05$) in the prevalence of *C. burnetii*. However, no significant association was found between risk factors and infection due to *C. abortus.* This study's findings suggest that antioxidant treatments or vaccination strategies can be beneficial to counteract the adverse effects of these infections on small ruminants.

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INTRODUCTION

Abortion in small ruminants is one of the leading causes of failure of sustainable small ruminants farming enterprises (Karakurt *et al.,* 2023). Several infectious diseases including salmonellosis, brucellosis, coxiellosis, chlamydiosis, and toxoplasmosis result in fetal loss in sheep and goats, thus, resulting in economic losses to the farmers (El Jai *et al.*, 2003; Hailat *et al*., 2022; Ahmed *et al.,* 2023). The flock prevalence of abortion was found 75.3% in small ruminants of Algeria (Kardjadj *et al.*, 2016), 10.2% in does and 12.1% in ewes of Morocco (Benkirane *et al.*, 2015), and 34 % in does and 48% in ewes of Netherland (Van den Brom *et al.*, 2012)

Chlamydia abortus and *Coxiella burnetii* are pathogens of zoonotic importance that can cause respiratory problems, hepatitis, endometritis, self-limiting flu-like disease, abortion, and the birth of weak or dead fetuses in humans, and animals (Roest *et al.*, 2012).

Chlamydia abortus is a gram-negative, obligate intracellular bacterium that causes abortion in small ruminants or the birth of a dead or weak fetus (Livingstone *et al.,* 2005). The exact pathogenesis of *C. abortus* is still unknown. Research studies have reported that the pathogen approaches the trophoblasts of fetal cotyledon through blood and causes inflammatory and necrotic changes in the placenta and cotyledon. The injury to the placenta causes hormonal imbalance and results in the expulsion of the fetus before the completion of the gestational period (Longbottom *et al.,* 2013).

Coxiella burnetii is also an obligate intracellular gram-negative bacterium that results in the abortion of small ruminants. The disease caused by this pathogen is known as Coxielliosis, also known as Q fever and cosmopolitan zoonosis. i.e., (Tesfaye *et al.,* 2020). In humans, the pathogen causes prolonged infection in the uterus, leading to severe endometritis (Sánchez *et al.,* 2006). In animals, the clinical manifestations of coxiellosis can vary widely, ranging from asymptomatic or mild flu-like symptoms to severe pneumonia or chronic infections leading to reproductive failure (El-Deeb *et al.,* 2019). After inhalation, the bacterium crosses the epithelial barrier of the respiratory system and invades alveolar macrophages. The bacterium resists digestion within the macrophage's phagolysosome and replicates to form highly resistant structures called phagolysosome-like parasitophorous vacuoles (PLPVs). Following replication within macrophages, *C. burnetii* can disseminate to various organs and tissues, leading to systemic infection (Van Schaik *et al.,* 2013).

Both of these infectious agents are isolated from the amniotic fluid, placental tissues, dead or aborted fetus, milk, and other body fluids and secretions of the infected animals and can live in the soil for several days (Hadush *et al.,* 2016). The infection is transmitted to other animals in the flock and humans through contact with the infected material, consumption of raw infected milk, or inhalation (Ullah *et al.,* 2019).

The term "antioxidant status" refers to the balance between the production of harmful reactive oxygen species (ROS) in the body and the body's ability to counteract their damaging effects through antioxidants (Talukder *et al.,* 2017). Antioxidants are the biomolecular substances that inhibit ROS production through oxidation reactions, protecting the cells and tissues from damage due to oxidative stress. Maintaining a healthy antioxidant status is crucial for overall health. In the context of abortion-causing bacteria, such as pathogens that cause septic abortions, disruptions in the antioxidant status can exacerbate cell injury (Da Silva *et al.,* 2019). These bacteria can induce inflammation and generate ROS thereby overwhelming the body's antioxidant defense. This oxidative stress can lead to cell injury and tissue damage, which may contribute to complications associated with abortion (El-Deeb *et al.,* 2019).

Serum biochemical changes are usually associated with cell injury. The cellular injury results in the serological alterations of specific biomarkers that can be detected and used to reflect tissue damage and inflammation (Mahboub *et al.,* 2013). Different serum biomarkers that are assessed in response to certain infections include acute phase proteins, globulins, liver function markers (e.g., ALT, AST, and ALP etc.) and renal function markers (e.g., creatinine, Blood Urea Nitrogen etc.) (Hashem *et al.,* 2020). These changes occur as a consequence of the body's response to a certain infection, involving both localized and systemic inflammation. Therefore, monitoring these serum markers is essential for early diagnosis and managing infections caused by abortion-associated bacteria (El-Deeb *et al.,* 2019). Besides routine microbial culturing and identification of *C. abortus* and *C. burnetii*, the infection can be analyzed through histopathology, ELISA, PCR, CFT, fluorescent antibody technique (FAT) and immunohistochemistry (IHC) (Rahman *et al.,* 2016; O'Neill *et al.,* 2018).

Abortion in animals is associated with different risk factors including the number of animals in the herd, season, age, sex, vaccination type, type of breeding system, previous treatment history, breeding type, farm hygiene, and mixed breeding farm (Rizzo *et al.,* 2016; Fayez *et al.,* 2021). However, research data is scanty about discussing the association of different risk factors with infection due to *C. abortus* and/or *C. burnetii* in animals. Furthermore, studies on changes in parameters such as oxidative stress caused by abortion-causing bacteria are also rare. Therefore, this study was designed to determine alteration in serum concentration of catalase enzyme and malondialdehyde as a marker of oxidative stress in seropositive and healthy small ruminants, and changes in serum protein concentration in seropositive and healthy animals along with the association between certain risk factors and seropositivity of any pathogen under study.

MATERIALS AND METHODS

Study area: The sampling was performed in the district Rajanpur (Punjab), Pakistan located at 29°6'15" N 70°19'29" E geographical coordinates. This district's sheep and goat population is 0.39 million and 0.633 million, respectively (Malik *et al.,* 2016). The average annually recorded temperature of Rajanpur is 31.39°C i.e., 10% higher than the average recorded temperature of Pakistan and the average precipitation level is 22.4 millimeters annually (Zulfiqar *et al.,* 2020).

Animal inclusion criteria and sample size: The antibodies against *C. abortus* are detectable in serum within a month after abortion (Longbottom *et al.,* 2013). Keeping in view this fact, serum samples were collected from only freshly aborted animals during the study period (n = 168). The information regarding different risk factors such as species, age, flock type, flock size, and pregnancy stage was obtained from owners and recorded on a predesigned proforma.

Blood sample collection and serum separation: A total of 168 blood samples were collected from the aborted sheep (n = 84) and goats (n = 84) from October 2021 to March 2022. The blood samples were collected aseptically from the animal's jugular vein and put into a vacutainer containing the sterile gel clot activator. The samples were centrifuged at 6000 rpm for 15 minutes, and the supernatant (serum) was separated into the serum cup. The serum samples were stored at -20°C until further serological analysis.

Enzyme-linked Immuno Sorbent Assay (ELISA) for determination of seropositive animals: Antibodies in these serum samples against *C. abortus* and *C. burnetii* were detected using commercial ELISA kits i.e., ID Screen® Chlamydophila abortus indirect multi-species (CHLMS-MS, Grabels, France) and ID Screen® Q Fever Indirect Multi-species (FQS-MS, Grabels, France). The manufacturer's instructions were followed to perform ELISA and the sample positive percentage was calculated using the formula given in the kit's manual.

Sample selection for Biochemical analysis: The samples from sheep and goats that were positive for the presence of antibodies against either *C. abortus* or *C. burnetii*, as well as those collected from clinically healthy seronegative animals (10 from each specie) for comparison of serum biochemical parameters, were included for further lab analysis.

Oxidative stress assessment: The enzyme activity of catalase from the selected serum samples was determined following the protocol described by Hadwan and Abed (2016). Briefly, 100µL of serum was mixed with 1000µL of 20mM hydrogen peroxide solution (prepared in 50mM sodium-potassium phosphate buffer pH 7.4) and incubated at 37°C for 3 minutes. Then, 4000µL of ammonium molybdate (32.4 mmol/l) was added to the reaction mixture and incubated at room temperature for 2 minutes. For each serum sample, the control test was also performed in which 1000µL of distilled water was added in place of H_2O_2 while keeping the rest of the procedure as same. The standard test for each set of experiments was performed by adding 100µL of distilled water instead of serum while the rest of the procedure was the same. The absorbances of the test, control test, and standard test solutions were determined spectrophotometrically at 374nm and catalase activity was determined by using the following formula:

$$\text{Catalase activity (kU)} = \frac{2.302}{t} \times \left[Log \frac{S^{\circ}}{S-M}\right] \times \frac{Vt}{Vs}$$

Where t is time, S° is the absorbance of standard test solution, S is the absorbance of test solution, M is the absorbance of the control test, Vt is the volume in a test tube, and Vs is the volume of the sample.

The serum concentration of malondialdehyde (MDA) as a biomarker of oxidative stress in control and test group animals was determined following the protocol of Feldman (2004). Briefly, different concentrations (0, 0.625, 1.25, 2.5, 5, 10, 50, and 100µM) of standard 1,1,3,3-tetramethoxypropane (TMP) were prepared in distilled water. 200µL of the ice-cold 10% trichloroacetic acid solution was added to the 100µL of each standard solution and serum sample in a 1.5mL centrifuge tube. The centrifuge tubes were then incubated for 15 minutes on ice and then centrifuged at 2200 ×g for 15 minutes at 4°C. The 200µL of supernatant was added to the 200µL of 0.67% thiobarbituric acid and incubated in the boiling water bath for 10 minutes. The samples were cooled down to room temperature and the absorbance of coloured product was recorded at 532nm. The concentration of MDA was determined through the equation of linear regression.

Biochemical analysis: The biochemical analysis was performed using a biochemistry analyzer (OPTIZEN, Model: 1412HF, South Korea) and commercially available kits to determine the serum concentration of total proteins (Liquick-TOTAL PROTEIN, Bioactiva diagnostic, Germany) and albumin (Liquik-ALBUMIN, Bioactiva diagnostic, Germany). The globulin concentration in all samples included in the study was determined by subtracting the value of serum albumin in a sample from the serum total protein concentration and the albumin to globulin ratio of each sample was calculated by dividing the concentration of albumin by the concentration of globulin.

Impact of risk factors: The information related to the risk factors was collected from the owners of the small ruminants and recorded on the pre-designed history record Performa. The risk factors included in the study were: the species of animal (sheep or goat), age of the animal (1-2 years or >2 years), flock type of the animal (nomadic or rural), flock size of the animal (<100 or >100), and pregnancy stage (<3 months i.e., Ist trimester or >3 months i.e., 2nd or 3rd trimester) of the animal.

Statistical analysis: Statistical analysis was performed by using IBM SPSS Statistics 21® for Windows.

1) The results of oxidative stress assessment and biochemical analysis of seropositive animals were compared with those of healthy animals by applying a one-way analysis of variance (ANOVA).

2) The chi-square test was applied to determine any association between the seropositivity of a disease and certain risk factors. A p-value of less than 0.05 was considered significant.

RESULTS

Seropositivity of *C. abortus* and *C. burnetii*: Among the total 168 samples, the number of positive samples for the presence of antibodies against *C. abortus* and *C. burnetii* were 8 and 32, respectively. Among goats (n=84), the number of goats infected with *C. abortus* and *C. burnetii* was 6 and 21, respectively. However, among sheep (n=84), the number of sheep infected with *C. abortus* and *C. burnetii* was 2 and 11, respectively. The results of seropositivity of *C. abortus* and *C. burnetii* are graphically presented in Fig. 1.

Oxidative stress assessment: In healthy, *C. abortus*, and *C. burnetii* seropositive animals the catalase concentration was 2.65±0.55 i.e., 1.32±0.21, and 1.45±0.33, respectively whereas that of MDA was 1.25±0.58, 2.89±0.70**,** and 2.91±0.93, respectively (Fig. 2). The catalase concentration in serum samples of sheep and goats seropositive for *C. abortus* and *C. burnetii* was significantly (P<0.05) lower than the catalase concentration in the serum of healthy animals (Fig. 3 A). Conversely, the concentration of MDA in the serum of seropositive sheep and goats was significantly (P<0.05) higher compared to the healthy animals (Fig. 3 B). The concentration of catalase and MDA did not differ significantly (P>0.05) between the animals infected with *C. abortus* and *C. burnetii*. The mean±SD of catalase and MDA concentration in infected and healthy sheep and goats is shown in Table 3.

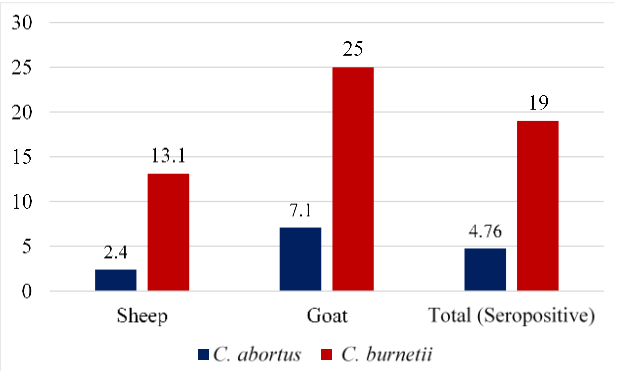

Fig. 1: Seropositive percentage (%) of *C. abortus* and *C. burnetii* in small ruminants of district Rajanpur.

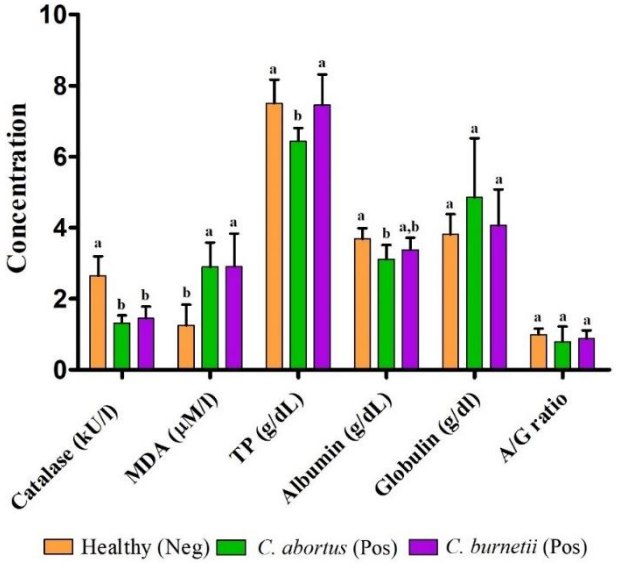

Fig. 2: Mean±SD serum concentration of catalase, MDA, TP, albumin, globulin, and A/G ratio in healthy, *C. abortus* (Pos) and *C. burnetii* (Pos). Different alphabets (a,b) on bars indicate that a significant difference (P<0.05) exists between healthy and infected animals.

Serum proteins assessment: The mean concentration of total proteins in all *C. abortus* positive animals was significantly lower (P<0.05) than the concentration of total proteins in healthy animals. The mean concentration of total proteins in *C. burnetii* infected animals did not differ significantly from that of healthy animals.

The mean concentration of albumin was significantly lower (P<0.05) in the *C. abortus* and *C. burnetii* positive sheep compared to the healthy sheep. However, in comparison to the healthy goats, the mean concentration of albumin was significantly lower (P<0.05) in *C. abortus* positive goats only and did not differ significantly (P>0.05) between *C. burnetii* goats and healthy goats.

The concentration of globulin was significantly higher (P<0.05) in *C. burnetii* positive sheep compared to the healthy sheep and did not differ significantly (P>0.05) between *C. abortus* positive and healthy sheep. The concentration of globulin in healthy, *C. abortus* and *C. burnetii* positive goats did not differ significantly (P>0.05).

The mean albumin-globulin (A/G) ratio in all *C. abortus* and *C. burnetii* positive animals did not differ significantly (P>0.05) from the A/G ratio of healthy animals. The concentration of the serum proteins was expressed as mean ± standard deviation (SD) of healthy and infected animals as shown in Table 4.

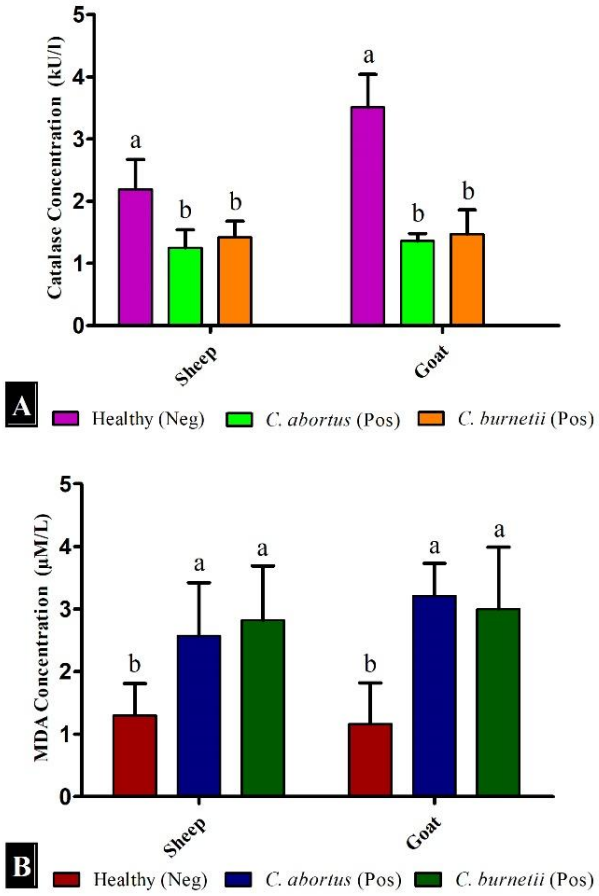

Fig. 3: Mean±SD serum concentration of Catalase (A) and MDA (B) in sheep and goats. Different alphabets (a,b) on bars indicate that a significant difference (P<0.05) exists between healthy and infected animals.

Association of risk factors with occurrence of *C. abortus* and *C. burnetii* infection: The positivity of *C. burnetii* was significantly higher (P<0.05) in goats (n=21/84) compared to sheep (n=11/84). Similarly, the positivity of *C. burnetii* was significantly higher (P<0.05) in nomadic flocks (n=28/113) than in rural flocks (n=4/55). In the case of *C. abortus*, the association between the risk factors and infection was not found. The results of the association of risk factors with the occurrence of *C. abortus* and *C. burnetii* infection are shown in Tables 1 and 2, respectively.

DISCUSSION

This research showed that the overall seropositivity of *C. abortus in District Rajanpur* was 4.76% (Table 1). Similarly, the 4.2% seroprevalence of *C. abortus* in small ruminants of Jhang was reported (Zeeshan *et al.*, 2023). However, another study in Pakistan reported a 37.7% prevalence of *C. abortus* in small ruminants of Sindh (Memon *et al.*, 2022). Previous studies reported that the positivity of *C. abortus* in small ruminants of different countries such as Saudi Arabia, Iran, Libya, and UAE was 19.4, 25.6, 12.2, and 19.6%-24%, respectively (Hussein *et al.*, 2008; Esmaeili *et al.*, 2015; Elzlitne and Elhafi 2016; Zaher *et al.*, 2017). The difference in findings might be attributed to differences in the geographical location of the study, climate, and pathogen identification techniques e.g., ELISA, PCR etc (Zeeshan *et al.*, 2023).

Table 1: Seroprevalence of *C. abortus* in small ruminants and associated risk factors

Risk factor	Category	Positive/ Total	Positive %	Odd-Ratio	95% CI	Chi-sq value	Sig.
Species	Sheep	2/84	2.4[a]	0.48	0.14- 1.62	2.202	0.14
	Goat	6/84	7.1[a]	1.55	1.04 –1.93		
Age	1-2 years	5/87	5.5[a]	1.22	0.69 –2.13	0.386	0.53
	>2 years	3/81	3.7[a]	0.76	0.31 –1.91		
Flock type	Nomadic	3/113	2.7[a]	0.54	0.22– 1.34	3.379	0.06
	Rural	5/55	9.1[a]	2.00	0.12 –3.59		
Flock size	< 100	2/60	3.3[a]	0.69	0.20 –2.33	0.420	0.86
	>100	6/108	5.6[a]	1.17	0.78 –1.79		
Pregnancy stage	>3 month	3/103	2.9[a]	0.60	0.24 –1.48	2.007	0.15
	<3 month	5/65	7.7[a]	1.67	0.94 –2.95		

[a,b] Significant differences exist between the two categories of same variable (P<0.05).

Table 2: Seroprevalence of *C. burnetii* in small ruminants and associated risk factors

Variable	Category	Positive/Total	% Positive	Odd-Ratio	95% CI	Chi-sq value	Sig.
Species	Sheep	11/84	13.1[a]	0.64	0.39-1.06	3.86	0.04
	Goat	21/84	25.0[b]	1.42	1.04-1.93		
Age	1-2 year	16/87	18.4[a]	0.96	0.65-1.04	0.50	0.82
	>2 years	16/81	19.8[a]	1.05	0.71-1.54		
Flock type	Nomadic	28/113	24.8[a]	1.40	1.16-1.68	7.35	0.00
	Rural	4/55	7.3[b]	0.33	0.13-0.86		
Flock size	< 100	8/60	13.3[a]	0.65	0.35-1.24	1.97	0.16
	>100	24/108	22.2[a]	1.21	0.96-1.54		
Pregnancy stage	>3 month	16/103	15.5[a]	0.78	0.54-1.13	2.13	0.14
	<3 month	16/65	24.6[a]	1.38	0.92-2.10		

[a,b] Significant differences exist between the two categories of same variable (P<0.05).

Table 3: Oxidative stress in *C. abortus* and *C. burnetii* infected sheep and goats

Variable	Specie	Healthy	C. abortus (Positive)	*C. burnetii* (Positive)
Catalase	Sheep	2.19±0.48[a]	1.25±0.29[b]	1.42±0.26[b]
	Goat	3.51±0.53[a]	1.36±0.12[b]	1.47±0.39[b]
MDA	Sheep	1.30±0.51[b]	2.57±0.85[a]	2.82±0.87[a]
	Goat	1.16±0.66[b]	3.21±0.52[a]	3.00±0.99[a]

Different superscripted alphabets ([a,b]) represent that a significant difference (P<0.05) exist between the columns

Table 4: Serum biochemistry of sheep and goats infected with *C. abortus* and *C. burnetti*

Variable	Species	Healthy	*C. abortus* (Positive)	*C.burnetii* (Positive)
Total protein	Sheep	7.81± 1.12[a,1]	6.46±0.37[b,1]	7.40±0.64[ab,1]
	Goat	7.61±0.72[a,1]	6.42±0.40[b,1]	7.10±0.24[a,1]
Albumin (A)	Sheep	3.48±0.20[a,2]	2.86±0.09[c,2]	3.14±0.19[b,2]
	Goat	3.90±0.22[a,1]	3.38±0.43[b,1]	3.63±0.27[ab,1]
Globulin (G)	Sheep	3.60±0.34[b,1]	3.92±0.58[ab,1]	4.67±1.27[a,1]
	Goat	3.30±0.54[a,2]	3.71±0.58[a,2]	3.47±0.11[a,1]
A/G ratio	Sheep	0.80±0.03[a,2]	0.90±0.13[a,1]	0.71±0.19[a,2]
	Goat	1.13±0.36[a,1]	1.07±0.17[a,1]	1.05±0.98[a,1]

Different superscripted alphabets ([a,b]) represent that significant differences (P<0.05) exist between the columns whereas different superscripted numbers ([1,2]) represent that a significant difference (P<0.05) exists between the rows while keeping confidence interval as 95%.

The overall seropositivity of *C. burnetii* in our research study was 19.05% (Table 2) which is slightly higher than the previously reported prevalence in Punjab province i.e., 15% (Ullah *et al.,* 2019). The prevalence of *C. burnetii* was higher than the prevalence of *C. burnetii* in domestic animals of Bangladesh, i.e. 5.06% (Rahman *et al.*, 2016). The difference in the prevalence of *C. burnetii* could be associated with different factors such as environmental conditions, genetic diversity in animals, husbandry practices and geographical distribution of the pathogen (Ullah *et al.*, 2020).

The reported prevalence of *C. abortus* and *C. burnetii* in livestock herds led to economic losses to farmers and posed a severe public health concern. Through assessment and identification of ill animals through ELISA veterinary clinicians can manage the infected livestock herds more efficiently through isolation and medication of the diseased animals, therefore, limiting the transmission of pathogens from one flock to another.

The oxidative stress results in cellular injury and can be assessed by measuring the activity of different oxidants and antioxidants (Celi 2011). Lipids are particularly susceptible to oxidation, and measuring lipid peroxidation products such as MDA can serve as a potential biomarker for oxidative stress (Ayala *et al.*, 2014). Similar to our findings, an increase in MDA concentration and decrease in catalase concentration due to infectious of *C. abortus* and *C. burnetii* in does and ewes (Table 3) have been previously reported by Balikci *et al.* (2013) and El-Deeb *et al.* (2019). Increased levels of free radicals circulating in the body can lead to the overproduction of MDA (El-Deeb and Tharwat, 2015). However, Balikci *et al.* (2013) studied abortion due to Border Disease Virus (BDV) in goats only and biomarkers other than MDA and catalase were also studied. The low levels of catalase enzymes found in infected animals could be due to their depletion in response to oxidative stress (El-Deeb *et al.*, 2019). If these enzymes fail to eliminate harmful radicals, the oxidation rate will exceed the antioxidation rate, ultimately leading to oxidative stress (Balikci *et al.*, 2013). Thus, releasing oxygen-free radicals due to bacterial infection or reduced antioxidant levels is a common cause of lipid peroxidation leading to cell injury (Gupta *et al.*, 2014) and necrosis of placentomes, therefore, resulting in the abortion of the fetus

(Al-Shaeli *et al.,* 2020; Caspe *et al.,* 2022). For veterinary practitioners, this research underlines the potential benefits of incorporating antioxidant supplements such as Vitamin E and Selenium as part of the treatment for infected animals.

In this study, a decrease in the serum total protein and albumin levels was observed in the *C. abortus* and *C. burnetii* infected animals (Table 4). Similarly, Mahboub *et al.* (2013) and Fayez *et al.* (2021) reported a decrease in total protein and albumin in the animals suffering from Q-fever or chlamydiosis. Previous studies on biochemical changes due to abortion-causing pathogens have indicated that the decline in total protein and albumin might have occurred due to hepatic damage. The burst of liver cells results in the release of certain liver-specific enzymes (AST and ALT) which gain access to the blood and thus their level rises in the serum (Al-Hussary and Al-Zuhairy 2010; Amany *et al.,* 2010). The lower level of proteins and albumin may also be associated with damage to the renal glomerulus, thus leading to the excessive excretion of the proteins and albumin in the urine (Hammouda *et al.,* 2006; Amany *et al.,* 2010). Similar to our findings, Mahboub *et al.* (2013) reported a rise in the serum globulin level in the sheep and goats infected with *Toxoplasma gondii* and *Brucella spp*. The increased level of globulin might be possible due to the adaptive immune response of the animal's immune system against abortion-causing pathogens resulting in the rise in level of the immunoglobulins (Abenga and Anosa 2005; Bauer *et al.,* 2021). The decreased level of total proteins and albumin in diseased animals might indicate poor nutrition managemental practices which impact growth and productivity (Zahid *et al.,* 2016). To mitigate the effects of nutritional causes of the disease, animals can be offered with protein-rich diet such as some concentrates that can boost the energy level of animals and strengthen their immunity to combat different infectious agents.

In this study, the chance of infection due to *C. abortus* in sheep and goats was the same (Fig. 1), however, the infection due to *C. burnetii* was higher in goats (25.0%) compared to sheep (i.e. 13.1%). Similar findings have been previously reported by Al-Qudah *et al.* (2004), Travnicek *et al.* (2002), and Aljumaah and Hussein (2012). The higher prevalence of *C. burnetii* in goats might be due to its environmental stability of the *C. burnetii,* capability to withstand high temperatures, dehydration, and disinfectants; the organism can endure in the environment for prolonged periods (Çekani *et al.,* 2008). The higher infection rate in goats compared to sheep also suggests species-specific vulnerability, requiring tailored preventive measures for each type of livestock.

Similar to the findings of this research, Zahid *et al.* (2016) also did not find any association between the infection due to *C. burnetii* and the age of the animals. In contrast to our findings, Esmaeili *et al.* (2015) reported a significant association between age and seropositivity due to *C. abortus* infection. This might be possible due to the presence of antibodies against *C. burnetii* in a population of animals, suggesting that they were exposed to the bacterium early in life and remained exposed throughout their lives (Astobiza *et al.,* 2012; Zahid *et al.,* 2016).

Similar to our findings, Esmaeili *et al.* (2021) reported that there is no significant association (P>0.05) between the flock type and the prevalence of *C. abortus.* Moreover, Muema *et al.* (2017) reported a higher prevalence of *C. burnetii* in nomadic flocks compared to other flock types. The findings align with earlier research indicating that grazing systems and those facilitating more significant casual interaction between infected and uninfected animals are associated with a higher likelihood of *C. burnetii* seropositivity (Alvarez *et al.,* 2012; Muema *et al.,* 2017).

In the case of flock size, our findings are supported by the results of Santos *et al.* (2012) and Fayez *et al.,* (2021) who found no significant difference between the flock size and infection due to *C. abortus* and *C. burnetii*. One explanation that has been proposed is that keeping a large number of sheep or goats in overcrowded flocks can hurt their welfare and hygiene. This, in turn, may increase the risk of transmitting these pathogens to the animals (Hireche *et al.,* 2016).

In the case of the pregnancy stage, our findings agree with the findings of Roest *et al.* (2020) and Esmaeili *et al.* (2021) who reported that there is no significant association between abortion due to *C. abortus* and *C. burnetii,* and the pregnancy stage. This outcome could be due to the coexistence of other abortion-causing agents, suchas *Brucella spp.*, *Neospora caninum,* and *Toxoplasma spp*., which are more prevalent in Pakistani flocks during late gestation i.e., 3rd trimester (Nasir *et al.,* 2012; Ali *et al.,* 2015; Ahmed *et al.,* 2016; Memon *et al.,* 2022).

This study demonstrated the prevalence of *C. abortus* and *C. burnetii* in the Rajanpur area of Punjab province of Pakistan.

Conclusions: It can be concluded that infection due to *C. abortus* or *C. burnetii* caused the alteration of antioxidant status and biochemical changes due to which abortion in small ruminants occurred. Based on the data of this study, further studies can analyze the potential advantages of using antioxidant medications or vaccination plans to eliminate the negative effect of these infections on small ruminants.

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Authors contribution: Muhammad Saadullah and Muhammad Tahir Meraj collected samples, performed sample analysis. Ishtiaq Ahmed, Aziz ur Rehman and Muhammad Kashif designed the research project, performed data analysis, wrote and proofread the manuscript. Muhammad Kamran Rafique and Muhammad Adnan Saeed helped in sample collection and analysis. Muhammad Shahbaz Yousaf performed assays for oxidative stress assessment. Arfa Tehreem assisted in laboratory work and the write-up of the manuscript. Shahid Nazir, Muhammad Kashif Saleemi and Tauseef UR Rehman performed statistical analysis of data and revised and proofread the manuscript.

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