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INVESTIGATING PREVALENCE, ANTIOXIDANT STATUS AND BIOCHEMICAL CHANGES IN *CHLAMYDOPHILA* INFECTION IN SHEEP

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ABSTRACT

A dangerous zoonotic bacterial infection that has a significant financial impact on the sheep industry is *Chlamydophila* spp. This investigation's aims were to determine the prevalence of *Chlamydophila* spp infection, study particular risk indicators, and assess the antioxidant status and biochemical changes of infected sheep. One hundred sheep were used in this study. Whole blood and serum samples were collected for laboratory analysis. ELISA and PCR had been employed for *Chlamydophila* spp diagnosis. The observed clinical findings of *Chlamydophila* spp infection in sheep were abortion, infertility, respiratory signs, conjunctivitis, diarrhea, and nervous manifestations. The seroprevalence of *Chlamydophila* spp infection was 2.20% (2/91) using ELISA. Of the studied sheep, 89 (89%) and 76 (76%) of 100 whole blood samples showed molecularly positive results for *Chlamydophila* spp, 16S rRNA and OMP2 genes, respectively. The infection rate of *Chlamydophila* spp had no significant variation by age and sex of infected sheep, but prevalence of *Chlamydophila* spp infection was significantly higher in autumn and winter, on farms, and in clinically diseased sheep. The concentration of TAC in the serum of *Chlamydophila* spp-infected sheep was significantly higher compared to healthy animals. The mean concentration of total protein, albumin, and GGT in *Chlamydophila* spp-infected sheep did not differ significantly from clinically healthy ones. It is essential to highlight the importance of efficient preventative and control measures throughout Egypt to reduce the prevalence of *Chlamydophila* spp infection in sheep.

Keywords: *Chlamydophila*, ELISA, PCR, Antioxidant status, Biochemical parameters

INTRODUCTION

Chlamydophila (previously Chlamydia) species (spp) is the cause of *Chlamydiosis,* which is a serious zoonotic bacterial disease and has a significant financial impact on sheep industries as well as public health (Polkinghorne *et al.*, 2009; Ababneh *et al.*, 2014 and Inchuai *et al.*, 2022). *Chlamydophila* spp are non-motile, Gram-negative coccoid, and obligate intracellular bacteria that belong to the *Chlamydiaceae* family and genus *Chlamydophila* (El-Berbawy and El-Khabaz, 2014; Chisu *et al.*, 2022 and Inchuai *et al.*, 2022). *Chlamydophila* spp have a distinct

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two-phase life cycle; they have two morphological forms: the metabolically active bacterium's reticulate body and the infectious form's elementary body (Inchuai *et al.*, 2022 and Rhawy and Al-Iraqi, 2024). It has been documented that two species of the genus *Chlamydophila*, *Chlamydophila abortus* and *Chlamydophila pecorum*, infect sheep (Lenzko *et al.*, 2011). *Chlamydophila abortus* is a major cause of late-term abortion (enzootic abortion of ewes), particularly in the two to three weeks prior to delivery, infertility, weak offspring, and infection in rams causing orchitis, seminal vesiculitis, and infertility, besides pneumonia (Li *et al.*, 2022 and Petridou *et al.*, 2022). On the other hand, chronic diseases in sheep, such as pneumonia, encephalomyelitis, polyarthritis, conjunctivitis, enteritis, metritis, mastitis, and infertility are associated with *Chlamydo-phila pecorum* (Ababneh *et al.*, 2014; Chahota *et al.*, 2015 and Bhardwaj *et al.*, 2017). The disease's effects worsen in sheep flocks under strict management during lambing (Malal *et al.*, 2020). The infection is primarily spread through uterine discharges, vaginal secretions, birth products, and abortion materials; beside, feces can also spread the disease. The resulting contamination provides the source of infection for susceptible hosts, such as sheep and humans (Malal *et al.*, 2020; Ali and Al-Bayati, 2022). *Chlamydophila* spp infection is entered into susceptible sheep by ingestion and inhalation of organisms from contaminated materials and environments in addition to the possibility of venereal transmission (Osman, 2007; Ahmed *et al.*, 2021 and Aldama *et al.*, 2022). Live lambs can act as carriers and pose a risk to naive sheep. The organisms may live in lymphoid tissues in the latent or quiet form in non-pregnant ewes until the onset of pregnancy (Ahmed *et al.*, 2021). Some species are known to be zoonotic pathogens, including *Chlamydophila abortus* (Chahota *et al.*, 2015 and Bhardwaj *et al.*, 2017). In humans, the symptoms of *Chlamydophila* infection can include subclinical infection, influenza-like symptoms, possibly life-threatening illnesses, and also abortion in pregnant women (Ababneh *et al.*, 2014; El-Berbawy and El-Khabaz, 2014 and Malal *et al.*, 2020). There are several methods for diagnosing *Chlamydophila* spp infection, including microscopic, bacterial isolation, serological, and molecular testing (Ababneh *et al.*, 2014 and Rhawy and Al-Iraqi, 2024). Although isolation of the bacterium from infected animals is the definitive diagnosis, this method is consistently impractical in diagnostic settings due to its high cost, time efforts, the organism's zoonotic risk, and inapplicability for epidemiological investigations (Malal *et al.*, 2020 and Ali and Al-Bayati, 2022). The most widely used method for epidemiological studies and diagnosis of *Chlamydophila* spp infection in sheep is serological testing, like enzyme-linked immunosorbent assay (ELISA) (Ababneh *et al.*, 2014 and Ali and Al-Bayati, 2022). It has been demonstrated that molecular methods, such as conventional polymerase chain reaction (PCR), can identify *Chlamydophila* spp infection in suspected sheep (Ababneh *et al.*, 2014). In field research, *Chlamydophila* spp is identified using conserved genes, such as 16S rRNA (Bhardwaj *et al.*, 2017 and Taheri *et al.*, 2021a). The outer membrane protein 2 gene (OMP2) amplification has been found to be an accurate tool for identifying sheep infected with *Chlamydophila abortus* (Ababneh *et al.*, 2014 and Taheri *et al.*, 2021a). The immune system is triggered in situations like stress, inflammation, chronic disease, and different infections, which leads to oxidative stress due to a rise in free radicals and an imbalance between the organism's oxidants and antioxidants, which arise from the oxidant system's degradation, causing oxidative stress (Akpınar *et al.*, 2024). Antioxidants are compounds that stop or slow down oxidation of an organism's oxidizable materials, such as proteins, lipids, carbohydrates, and DNA. Tissue damage happens when the

body does not produce enough antioxidants (Akpınar *et al.*, 2024 and Saadullah *et al.*, 2024). These microorganisms can overwhelm the body's antioxidant defenses by causing inflammation and producing reactive oxygen species (Saadullah *et al.*, 2024). These bacteria seriously impair the health of sheep by harming critical organs, including the liver, kidney, and heart. When these organs are damaged, biochemical changes occur, which raise specific liver enzymes and decrease the amount of liver-produced proteins (Zeeshan *et al.*, 2023 and Saadullah *et al.*, 2024). Given the financial significance of problems in sheep caused by *Chlamydophila* spp infection, the current study was conducted to explore the prevalence of *Chlamydophila* spp infection by using ELISA and PCR, identify certain risk factors, and assess sheep's antioxidant status and biochemical changes associated with *Chlamydophila* spp infection.

MATERIALS AND METHODS

1. Animals and Ethical Approval

The investigation was carried out from June 2023 to August 2024. A clinical examination, serological testing for *Chlamydophila* antibodies, and molecular analysis for *Chlamydophila* DNA were performed on one hundred sheep of different ages, sexes, and locations. Fifty sheep were tested from an Al-Minya Governorate farm with a history of infertility, stillbirth, and abortion for 5 years. Additionally, fifty individual sheep from various villages in Assiut Governorate were investigated at Veterinary Teaching Hospital, Assiut University's Faculty of Veterinary Medicine. The most studied sheep suffered from infertility, abortion, diarrhea, respiratory signs (purulent nasal discharge and cough), conjunctivitis, and nervous signs. Each sheep used in this investigation was handled in accordance with ethical guidelines. The study was approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Assiut University in Assiut, Egypt, and was given approval number 06/2025/0293.

2. Clinical examination

The clinical examination of the studied sheep followed guidelines by Jackson and Cockcroft (2002).

3. Sampling

After the examined sheep were appropriately restrained, 5 ml of whole blood was collected from their jugular vein and divided into two parts. One part (2 ml) was put into sterile ethylene diamine tetraacetic acid (EDTA) vacutainer tubes and kept at -20°C for subsequent extraction of DNA. The second part (3 ml) was placed into sterile plain vacutainer tubes free from anticoagulants and allowed to clot at room temperature. After clotting, it was centrifuged at 3000 r.p.m. for 20 minutes, and serum was carefully obtained and stored in separate tubes at -20°C for further serological investigation, antioxidant status, and biochemical parameter detection (Petridou *et al.*, 2022 and Saadullah *et al.*, 2024).

4. Serological diagnosis by ELISA

A commercially available ELISA kit (Sheep *Chlamydia* antibody ELISA kit, Sunlong Biotech, China) was used to analyze 91 serum samples for the presence of specific antibodies for *Chlamydophila* spp in accordance with directions provided by the manufacturer. The optical density values were measured using an ELISA reader (Sunrise absorbance reader, Tecan, Austria) set at 450 nm at the Molecular Biology Research Center, Assiut University.

5. Molecular diagnosis by PCR

5.1. Extraction of DNA

The bacterial DNA was extracted from 100 frozen whole blood samples using ABT genomic DNA mini extraction kit (Applied Biotechnology, Egypt) according to the manufacturer instructions. The extracted DNA was stored at -20°C until used.

5.2. Primers

The specifics of the selected primers (Metabion International AG, Germany) used in the current study for the 16S rRNA gene of *Chlamydophila* spp. The sequences of these primers were as follows: 16SIGF: 5′- GAT GAG GCA TGC AAG TCG AAC G -3′ and 16SIGR: 5′- CCA GTG TTG GCG GTC AAT CTC TC -3′ with amplified PCR product at 278 bp (Borel *et al.*, 2006), while sequences of OMP2 gene of *Chlamydophila abortus* were as follows: OMP-F: 5′- ATG TCC AAA CTC ATC AGA GGA G -3′ and OMP-R: 5′- CCT TCT TTA AGA GGT TTT ACC CA -3′ with amplified PCR product at 587 bp (Ababneh *et al.*, 2014).

5.3. Detection of 16S rRNA and OMP2 genes by PCR

A PCR can be constructed particularly to amplify 16S rRNA and OMP2 genes of *Chlamydophila* spp and *Chlamydophila abortus*, accordingly. DNA fragments of 278 bp were amplified using primer sets 16SIGF forward and 16SIGR reverse, whereas primer sets OMP-F forward and OMP-R reverse were used for amplification of 587 bp (Rhawy and Al-Iraqi, 2024). In this study, polymerase enzyme and DNTPs were supplied using ABT red master mix (2X) (Applied Biotechnology, Egypt). PCR was performed using a PCR thermocycler (Techine, UK) and the following reagents: 16 µl total, which included 8 µl of ABT red master mix (2X), 0.5 µl of each primer (5 pmol), 3 µl of DNA template, and 4 µl of PCR molecular grade water. Ultimately, the primer set (16SIGF/16SIGR) was thermally cycled. Denaturation began with a 10-minute period at 94°C. There were forty cycles of denaturation at 94°C for one minute, annealing at 60°C for one minute, and extension at 72°C for one minute. The final extension lasted ten minutes at 72°C. The primer set (OMP-F/OMP-R) was analyzed according to thermal cycling conditions similar to the primer set (16SIGF/16SIGR), except the initial denaturation step was 94°C for 2 minutes and the annealing step was 54°C for 1 minute.

5.4. Analysis and identification of PCR products

Seven microliters of amplified DNA product were added to visualize the reaction. The amplicons were subjected to 75 minutes of 1.5% agarose gel electrophoresis stained with ethidium bromide (10 mg/ml) at 90 V and 155 mA before becoming visible with a gel UV transilluminator (Syngene, UK). The amplicon size was measured using a 100-bp DNA ladder.

6. Sample selection for antioxidant status and biochemical parameters

The antioxidant status and biochemical parameters studies used 75 samples positive for *Chlamydophila* spp DNA (59 in the reproductive problems group, 15 in the respiratory distress, enteritis, and nervous signs group) and seven samples negative for *Chlamydophila* spp DNA as a control group (clinically healthy).

6.1. Antioxidant status evaluation

Total Antioxidant Capacity (TAC) was determined with a colorimetric test kit (Biodiagnostic, Egypt).

6.2. Biochemical parameter analysis

The biochemical examination was performed using a biochemistry analyzer (Mecasys, Korea) and commercially available kits to assess serum content of total proteins & albumin (Spectrum, Germany) and serum liver enzymes such as gamma-glutamyl transferase (GGT) (Spectrum, Germany) according to the manufacturer's directions.

7. Statistical analysis

Statistical analysis was performed by using the statistical package for social sciences (SPSS) version 16 software (2007):

- The clinical, serological, molecular, and epidemiological results were obtained and analyzed using the Chi-square test of independence.

- Duncun was used to evaluate antioxidant status and biochemical parameters between molecularly positive and clinically healthy sheep.

RESULTS

1. Clinical findings of investigated sheep

The observed clinical features of *Chlamydophila* spp infection in sheep used in this study were abortion, infertility, respiratory signs (nasal discharge and cough), conjunctivitis, diarrhea, and nervous manifestations (ataxia and circling) (Table 1).

Table 1: Clinical signs of *Chlamydophila* spp infection in examined sheep

Clinical findings	No. of assessed investigated sheep	No. of 16S rRNA gene positive sheep (%)	No. of OMP2 gene positive sheep (%)	P-value
Reproductive disorders (abortion & infertility)	70	69 (98.57%)	58 (82.86%)**	
Respiratory signs (nasal discharge & cough), conjunctivitis, diarrhea and nervous manifestations (ataxia & circling)	21	19 (90.48%)	17 (80.95%)	
Clinically healthy	9	1 (11.11%)	1 (11.11%)	0.000**
Total	100	89 (89%)	76 (76%)	

**Highly significant increase at p<0.001 (0.000).

2. Serological diagnosis by ELISA

In examined sheep, the overall sero-prevalence of *Chlamydophila* spp infection was 2.20% (2/91) using ELISA (Table 2).

3. Molecular detection of *Chlamydophila* spp. and *Chlamydophila abortus* infections by PCR

To analyze DNA samples, PCR was utilized to generate the necessary bands at 278 bp of the 16S rRNA gene of *Chlamydophila* spp (Figure 1A) and to produce the particular diagnostic bands at 587 bp of the OMP2 gene of *Chlamydophila abortus* (Figure 1B). Of 100 whole blood samples from sheep under investigation, 89 (89%) and 76 (76%) had molecularly positive results for 16S rRNA and OMP2 genes, respectively (Table 2). The findings indicated that the 16S rRNA gene was more frequently determined than the OMP2 gene of *Chlamydophila* spp, and thirteen samples tested positive for the 16S rRNA gene but negative for the OMP2 gene (Table 3).

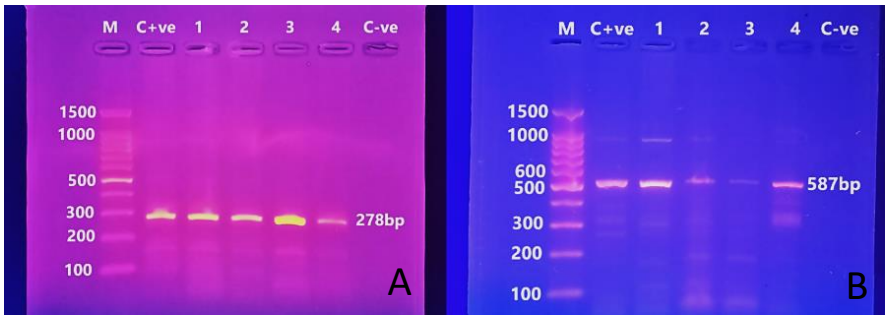

Figure 1: Agarose gel electrophoresis of PCR following 16S rRNA (A) and OMP2 (B) genes amplification of *Chlamydophila* spp and *Chlamydophila abortus* infections in studied sheep, respectively. Line M: DNA ladder 100 bp, line C+ve: Control positive sample; lines 1, 2, 3, and 4: Positive samples and line C-ve: Control negative.

Table 2: Prevalence of *Chlamydophila* spp infection in investigated sheep by serological and molecular methods

Test		No. of investigated sheep	No. of positive (%)	No. of negative (%)	P-value
ELISA		91	2 (2.20)	89 (97.80)	0.000**
PCR	16S rRNA gene	100	89 (89)	11 (11)	
	OMP2 gene	100	76 (76)	24 (24)	

**Highly significant increase at p<0.001 (000).

Table 3: Comparison between 16S rRNA and OMP2 genes results in detection of *Chlamydophila* spp infection in investigated sheep

		OMP2 gene		Total
		Positive	Negative	
16S rRNA gene	**Positive**	76	13	89
	Negative	0	11	11
Total		76	24	100

4. Possible risk factors

This study emphasized a few risk factors that affect the prevalence of *Chlamydophila* spp infection in sheep, including age, sex, season, housing system, and health status (Table 4). The infection rate of *Chlamydophila* spp had no significant effect by age and sex of infected sheep. However, the prevalence of *Chlamydophila* spp infection was considerably higher in autumn and winter, on farms, and in clinically diseased sheep (Table 4).

5. Antioxidant status evaluation

The value of the TAC marker of *Chlamydophila* spp-infected and clinically healthy sheep is presented in Table (5). The concentration of TAC in serum of *Chlamydophila* spp-infected sheep was significantly (P<0.01) higher compared to healthy ones.

6. Biochemical parameter analysis

The values of total protein, albumin, and GGT markers of *Chlamydophila* spp-infected and clinically healthy sheep are shown in Table (5). The mean concentration of total protein, albumin, and GGT in *Chlamydophila* spp-infected sheep did not differ significantly (P>0.05) from clinically healthy ones.

DISCUSSION

Chlamydophila spp is a significant bacterial pathogen that causes harmful infections in both animals and humans (Bhardwaj *et al.*, 2017). Abortion, infertility, respiratory problems (cough and nasal discharge), conjunctivitis, diarrhea, and neurological manifestations (ataxia and circling) were observed clinical findings with suspected clinical cases of *Chlamydophila* spp in the present study. These signs resembled those reported in previous investigations by Osman (2007); Polkinghorne *et al.* (2009); El-Berbawy and El-Khabaz (2014); Bhardwaj *et al.* (2017) and Ahmed *et al.* (2021).

Using ELISA, the overall seroprevalence of *Chlamydophila* spp infection in the current investigation was 2.20% (2/91). However, this finding was lower than that of El-Berbawy and El-Khabaz (2014); Fayez *et al.* (2021); Li *et al.* (2022) and Nogarol *et al.* (2024), who used ELISA to identify antibodies against *Chlamydophila* spp in 18.48%, 10.89%, 7.93%, and 50% of serum samples from sheep under study, respectively. The variability in results can be explained by differences in husbandry, management practices, sample numbers, and geographies.

Table 4: Correlation between *Chlamydophila* spp infection in studied sheep and possible risk factors according to PCR result (depending on 16S rRNA gene)

Variable		No. of examined sheep	PCR		P-value
			No. of positive (%)	No. of negative (%)	
Age	6 months - 1year	7	6 (85.71)	1 (14.29)	0.70
	> 1 - 2 years	75	66 (88)	9 (12)	
	> 2 - 3 years	18	17 (94.44)	1 (5.56)	
	Total	100	89 (89)	11 (11)	
Sex	Male	46	41 (89.13)	5 (10.87)	0.97
	Female	54	48 (88.89)	6 (11.11)	
	Total	100	89 (89)	11 (11)	
Season	Summer	37	27 (72.97)	10 (27.03)	0.001**
	Autumn	2	2 (100)	0 (0)	
	Winter	10	10 (100)	0 (0)	
	Spring	51	50 (98.04)	1 (1.96)	
	Total	100	89 (89)	11 (11)	
Housing system	Farm	50	49 (98)	1(2)	0.004**
	Household	50	40 (80)	10 (20)	
	Total	100	89 (89)	11 (11)	
Health status	Clinically diseased	91	88 (96.70)	3 (3.30)	0.000**
	Clinically healthy	9	1 (11.11)	8 (88.89)	
	Total	100	89 (89)	11 (11)	

No significant variation at $p<0.05$. **Highly significant differences at $p<0.01$ (0.004). **Highly significant variation at $p<0.01$ (0.001&0.000).

Table 5. Oxidative stress and serum biochemistry in *Chlamydophila* spp-infected sheep

Variable	Clinically healthy group	Reproductive disorders group	Respiratory distress, enteritis and nervous signs group	P-value
TAC	0.61 ± 0.16^{b}	0.88 ± 0.2^{a}	0.76 ± 0.24^{ab}	0.002
Total protein	5.65 ±0.6	6.29 ±0.99	6.08 ±1.37	0.281
Albumin	2.62 ±0.3	3.01 ±0.9	2.47 ±0.52	0.060
GGT	22.13 ±6.7	29.13 ±2.3	31.66 ±4.5	0.500

^{a,b} Highly significant differences exist between the infected and healthy animals ($P<0.01$).

In molecular identification utilizing PCR, *Chlamydophila* spp and *Chlamydophila abortus* had been verified in 89/100 (89%) and 76/100 (76%) using 16S rRNA and OMP2 genes, respectively. The high rate of *Chlamydophila* spp infection in studied sheep using PCR was almost identical to findings of a prior investigation by Lenzko *et al.* (2011), who observed that 78% of assessed sheep had *Chlamydophila* spp infection by PCR. This result could be explained by the fact that *Chlamydophila abortus* infects sheep for at least two to three years (Ahmed *et al.*, 2021), and most investigated infected sheep were in contact with animals and suffered from infertility and stillbirth. Our findings were consistent with other researchers, such as Rhawy and Al-Iraqi (2024), who reported that the percentage of positive samples for molecular diagnosis of *Chlamydophila abortus* in sheep was 11.46% for the 16S rRNA gene and 4.45% for the OMP2 gene. According to our research, the 16S rRNA gene was identified in sheep under investigation more prevalent than the OMP2 gene of *Chlamydophila* spp. The reasons for this finding may be attributed to the 16S rRNA gene being preferred in PCR diagnosis, because it keeps its

sequences for a single species and varies eventually over millions of years, and its size is also suitable for determining genetic species (Rhawy and Al-Iraqi, 2024). All these factors support the 16S rRNA gene's suitability for this and other investigations. This gene, which expresses 90% of its identity and sets it apart as a bacterium from other *Chlamydophila*-like species, was the basis for the 1999 reclassification of the *Chlamydiales* family (Everett and Andersen, 1999). Furthermore, *Chlamydophila abortus* only possesses one copy of the 16S rRNA gene, as opposed to two copies in other *Chlamydophila* species. This gives the gene a distinct advantage over other genes used for diagnosis (Thomson *et al.*, 2005). In our investigation, 13 sheep samples had positive to 16S rRNA gene, but negative to OMP2 gene results. This phenomenon could be because the animals were infected with other *Chlamydophila* spp, rather than *Chlamydophila abortus*.

PCR and ELISA results in the present study do not match, because many blood samples positive for PCR had negative ELISA results. A possible explanation is that these animals may be in the early or late stage of infection, when there are insufficient antibodies to detect *Chlamydophila* spp infection using serological testing.

Various risk factors, such as the age and sex of sheep under examination, seasonal alterations, the housing system, and health status were studied for their potential to interact with the infection rate of *Chlamydophila* spp. Regarding age susceptibility, there was no statistically significant distinction in the rate of *Chlamydophila* spp infection between age groups of sheep involved in the research. This outcome corroborated the findings of Selim *et al.* (2018); Sun *et al.* (2020); Zeeshan *et al.* (2023) and Saadullah *et al.* (2024), who concluded that there was no statistically significant fluctuation in the prevalence of *Chlamydophila* spp infection throughout all age groups of sheep under observation. Our results imply that the studied sheep were equally susceptible to acquire an infection with *Chlamydophila* spp. In terms of sex vulnerability, there was no statistically significant disparity in the rate of *Chlamydophila* spp infection between male and female sheep under study. Our results were in concurrence with those of Qin *et al.* (2014) and Fayez *et al.* (2021), who did not observe any appreciable differences in the rate of *Chlamydophila* spp infection by animal sex. Our outcomes may indicate that sex is not a major contributory factor for sheep infection with *Chlamydophila* spp (Qin *et al.*, 2014), as both male and female sheep were equally vulnerable to this non-sex-related disease. Assessing seasonal fluctuations and the rate of *Chlamydophila* spp infection, there was a substantial rise in the prevalence of *Chlamydophila* spp infection in the autumn and winter seasons (100%) compared to the spring (98.04%) and summer (72.97%) seasons. Our findings were consistent with those of Fayez *et al.* (2021), who found that *Chlamydophila abortus* was substantially more common in sheep flocks during winter than throughout summer. Moreover, Taheri *et al.* (2021b) found that summer had the lowest prevalence of *Chlamydophila abortus,* and autumn had the greatest. Our findings may be explained by the increased ability of *Chlamydophila* spp infection to withstand colder temperatures. Furthermore, a humid environment is conducive to *Chlamydophila* spp survival (Fayez *et al.*, 2021). Based on the housing system, this study found that 98% of farms and 80% of households rearing sheep had significant variations in the prevalence of *Chlamydophila* spp infection. These findings showed that the sheep's housing system was a risk contributor to *Chlamydophila* spp infection, and that ongoing exposure to infection by sheep was the reason for the high rate of *Chlamydophila* spp infection on the farm.

Likewise, close contact between infected sheep and non-infected sheep may facilitate the spread of *Chlamydophila* spp infection. In our investigation, the prevalence of *Chlamydophila* spp infection by health status was 96.70% of clinically diseased sheep and 11.11% of clinically healthy ones, with significant differences. The findings were consistent with those of El-Berbawy and El-Khabaz (2014), who found the infection rate was higher in sheep exhibiting clinical signs of *Chlamydophila* spp infection (22.22%) than in those without health problems (5%). The increased frequency of *Chlamydophila* spp infection in clinically diseased sheep may be caused by a number of reasons, including stress, animal resistance, and the quantity of bacteria spread to animals.

The activity of various oxidants and antioxidants can be used to measure oxidative stress, which causes cellular damage (Saadullah *et al.*, 2024). When *Chlamydophila* spp infection occurs, inflammatory reactions trigger activation of macrophages and polymorphonuclear leukocytes, which raises production of reactive oxygen species (ROS) and causes oxidative stress (Akpınar *et al.*, 2024). In this study, it was determined that TAC concentration was statistically significantly higher in the *Chlamydophila* spp-infected sheep group. This result contradicted previous studies by Ahmadi *et al.* (2018); Liu *et al.* (2021) and Akpınar *et al.* (2024), who concluded that TAC concentration was lower in the *Chlamydophila* spp-infected group. Our results could be explained by the fact that *Chlamydophila* spp causes abortion. In this case, a disruption in antioxidant status may exacerbate cell damage (Da Silva *et al.*, 2019). These bacteria have the ability to overcome the body's antioxidant defenses by causing inflammation and producing ROS. Cell injury and tissue damage brought on by this oxidative stress may exacerbate abortion-related problems (El-Deeb *et al.*, 2019).

Serum biochemical markers, including total protein, albumin, and GGT, were examined in sheep with and without *Chlamydophila* spp infection. In our findings, there was no significant difference ($P>0.05$) in the mean concentration of total protein and albumin between sheep infected with *Chlamydophila* spp and those clinically healthy. These findings were in conflict with previous investigations by Salman *et al.* (2020); Kifouly *et al.* (2024) and Saadullah *et al.* (2024), who reported that the mean concentration of total protein and albumin in all *Chlamydophila* spp-positive sheep was significantly lower than in healthy animals. The fact that our results showed no discernible difference in serum total protein and albumin levels between infected and healthy individuals with *Chlamydophila* spp infection may be because infection does not directly alter these proteins in a way that changes their bloodstream concentration. In the present study, GGT level in *Chlamydophila* spp-infected sheep did not differ significantly from clinically healthy ones. This outcome might be explained that GGT is an indicator of liver damage and may not be an effective marker for infection caused by *Chlamydophila* spp that mainly affects the reproductive system.

CONCLUSION

The present study identified *Chlamydophila* infection in sheep. *Chlamydophila* infection was associated with changes in antioxidant status and biochemical parameters in sheep. To reduce the adverse effects of *Chlamydophila* infection on animals' health, the results highlight the necessity of improved control of disease, screening programs, and increased awareness and preventive measures.

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دراسة مدى تواجد وحالة مضادات الأكسدة والتغيرات البيوكيميائية في عدوى الكلاميدوفيلا في الأغنام

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الكلاميدوفيلا هي عدوى بكتيرية ضارة حيوانية المنشأ تؤثر بشكل كبير على صناعة الأغنام اقتصاديا. تهدف هذه الدراسة إلى دراسة انتشار عدوى الكلاميدوفيلا وتحديد بعض عوامل الخطر وتقييم حالة مضادات الأكسدة والتغيرات البيوكيميائية لدى الأغنام المصابة. تم دراسة مائة رأس من الأغنام في هذه الدراسة. تم جمع عينات الدم الكامل والمصل للتحليل المعملى. تم استخدام الاليزا و تفاعل البلمرة المتسلسل لتشخيص الكلاميدوفيلا. كانت النتائج الاكلينيكية الملحوظة لعدوى الكلاميدوفيلا في الأغنام هي الإجهاض، العقم، أعراض تنفسية، التهاب الملتحمة، الإسهال وأعراض عصبية. بلغ معدل الانتشار السيرولوجى لعدوى الكلاميدوفيلا ٢.٢٠٪ (٩١/٢) باستخدام الاليزا من بين الأغنام المدروسة، أظهرت ٨٩ (٨٩٪) و ٧٦ (٧٦٪) من ١٠٠ عينة دم كامل نتائج إيجابية جزيئيًا لجينات 16S rRNA و OMP2 على التوالي. لم يُلاحظ اختلاف كبير في معدل الإصابة ببكتيريا الكلاميدوفيلا حسب عمر وجنس الأغنام المصابة؛ إلا أن معدل انتشار الإصابة بها كان أعلى بكثير في الخريف والشتاء، وفي المزارع، وفي الأغنام المصابة اكلينيكيا. كان تركيز TAC في مصل الأغنام المصابة ببكتيريا الكلاميدوفيلا أعلى بكثير مقارنةً بالأغنام السليمة. ولم يختلف متوسط تركيز البروتين الكلي والألبومين و GGT في الأغنام المصابة ببكتيريا الكلاميدوفيلا اختلافًا كبيرًا عن متوسط تركيزه في الأغنام السليمة اكلينيكيا. ولتقليل معدل انتشار الإصابة ببكتيريا الكلاميدوفيلا في الأغنام، من الضروري التأكيد على تطبيق تدابير وقائية ومكافحة فعّالة في جميع أنحاء مصر.