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Overview on Brucellosis in Camels

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ABSTRACT

A total of 270 dromedary dairy camels above five years old with a history of reproductive disorders, including abortion, repeated breeding, reduced milk production, and retained fetal membranes belonging to different localities at El-Sharqia Governorate, including Abu Kabir, Belbis, DeyarbNegm, EL Husseiniya, and Minya al-Qamh were employed in this study. Different risk factors associated with camel brucellosis were investigated in these camel's populations, these include the rearing of camels with different ruminant's species, addition of new animals, as well as lack of sanitary measures. The modified Rose Bengal Test and Complement Fixation Test revealed a prevalence of 40 (14.8%) and 32 (11.9%) respectively. The three districts with the highest prevalence of camel brucellosis were Belbeis (14.12%), EL Husseiniya (12.19%), and Minya al-Qamh (10.7%). Three (9.4%) *brucella* species were isolated out of 32 milk samples from serologically positive she-camels. Two isolates were identified as *B. melitensis* biovar 3 and one isolate was identified as *B. abortus* biovar1. *Brucella* strain DNA extracts from milk samples showed a specific amplicon of 731 bp specific for *B. melitensis* (18) and an amplicon of 498 bp specific for *B. abortus* (4), but no amplicon of 285 bp specific for *B. suis* could be detected after being subjected to PCR using the IS711 primer in this investigation. We concluded that, in this study, *Brucella meliteneis biovar 3* is a common field strain in camels. Oxidative stress biomarkers, malonaldehyde (MDA) were increased significantly ($P \leq 0.05$) in infected camel, whereas nitric oxide (NO) was moderate increase. Antioxidant enzymes like Glutathione Peroxidase enzyme (GPx) was significantly increase whereas catalase (CAT) was significantly reduced in infected camel. This study has confirmed our understanding of risk factors associated with camel brucellosis. The rearing of camels with different ruminant's species, the addition of new animals, and the lack of sanitary measures were the primary risk factors associated with the spread of brucellosis among dairy camels.

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INTRODUCTION

Camels are an essential domestic species for pastoralists in Egypt, who depend on animals for food security. Camels have a significant economic influence on farm animals **(ShahidNazir 2014).** Infected camels and their products could be a source of human brucellosis, leading to severe arthritis, fever, infertility, and in some cases, chronic infections subsequent misdiagnosis (**ShahidNazir, 2014;GutemaWegi, 2020)**. Milk is approved as the primary source of human infectivity **(Adamou Harouna, 2008).** The first case of camel brucellosis in Egypt was documented by **Ahmed (1939)**, and since that time, the prevalence of camel brucellosis has changed significantly over the past years. This can be explained based on the regular importation of camels into Egypt from several African countries. Owing to the practice of keeping camels in close contact with ruminants including sheep, goats, cattle, and buffaloes, particularly in large populations. Camels may be exposed to brucella infection which highlights the significance of the practice of mixing camels with other animal species either through grazing or sharing watering points. While camels can get Brucella infections from species that have already been demonstrated to be widely distributed in cattle, sheep, and goats, there is no specific Brucella species that exhibits a preference for camels **(Sayed et al. 2017)**. Despite not being known as the principal host for any Brucella species, camels can get infections from *Brucella abortus* and *Brucella melitensis* **(Musa and Shigidi, 2001).** Three Brucella species are potential causes of infection in camels, *B. abortus*, *B. suis* and *B. melitensis* **(Aman et al. 2020)**. The extent of brucellosis depends on the presence of the *Brucella* species in other animals that live nearby and on animal husbandry **(Musa et al. 2008).** The cornerstone for the control and eradication of Brucella infection is the diagnosis of brucellosis utilizing fluid samples, such as milk and blood samples **(Seleem et al. 2010).**

In view of the fact that none of the commonly used serological test can be regarded as most efficient serological test used for camels, a misguided determination may happen when conclusion is based on serology alone. Such variations and differences may be attributed to apparent discrepancies in the pathogenesis and progression of the brucellosis in camels (***Gwida et al.* 2012).** Clinical samples can be used directly for molecular identification of Brucella species without first isolating the bacterium. These methods can also be used to coordinate the results of phenotypic testing as described by **Bricker (2002).** Thus, characterization of *Brucella* DNA by PCR in clinical samples is considered a chosen tool for definitive diagnosis of brucellosis (**Ulu et al. 2013).**

The recognition of *Brucella* spp. in different farm animals and wildlife species emphasizes the role which these animals play in the transmission of the disease **(Machavarapu *et al*. 2019).**

Monitoring oxidative stress, blood biochemistry and antioxidant enzymes are essential tools to inferior the adverse impacts of oxidative stress in animals according to **Agrawal *et al*. (2003), Gomez *et al*. (2018)** and **Joshi *et al*. (2018),** free radicals alter steroidogenesis, apoptosis, lipid peroxidation, and folliculogenesis leading to disorders in embryo preimplantation and infertility in animals**.** Therefore, the estimation of blood biochemistry, oxidative stress and endogenous antioxidants are considered crucial factors in initiating different molecular mechanisms during infectious diseases **(Hussain *et al*. 2022).**

Due to inadequate surveillance programs for camel herds in several developing countries, the percentage of camels with *Brucella* spp. infection is rather high. Additionally, camel-raising countries severely restrict efforts to manage camel brucellosis **(Sprague *et al*. 2012).** Therefore, a variety of strategies should be employed to improve the epidemiological situation with regard to camel brucellosis and significantly reduce the disease's occurrence. Brucellosis in camel has received comparatively slight attention by means of milk as material for diagnosis of brucellosis. Therefore, the objectives of this study were to (i) Determine the important risk factors associ-

ated with camel brucellosis, that have an influence on the spread of infection. (ii) Identify *Brucella* species from She-camel's milk using bacteriological and molecular methods. (iii) Assessment of oxidative stress, and antioxidant profile in Brucella infected Camel .

2. MATERIALS AND METHODS:

2.1. Study Area and Animals

A total of 270 dromedary dairy camels older than five years old with a history of reproductive disorders, including abortion, repeated breeding, reduced milk production, and retained fetal membranes associated with risk factors were serologically, bacteriologically, and molecularly investigated for diagnosis of camel brucellosis. These camels belonged to different localities in El-Sharqia Governorate, including Abu Kabir, Belbeis, DeyerbNegm, EL Husseiniya and Minya al-Qamhas shown Fig. (1).

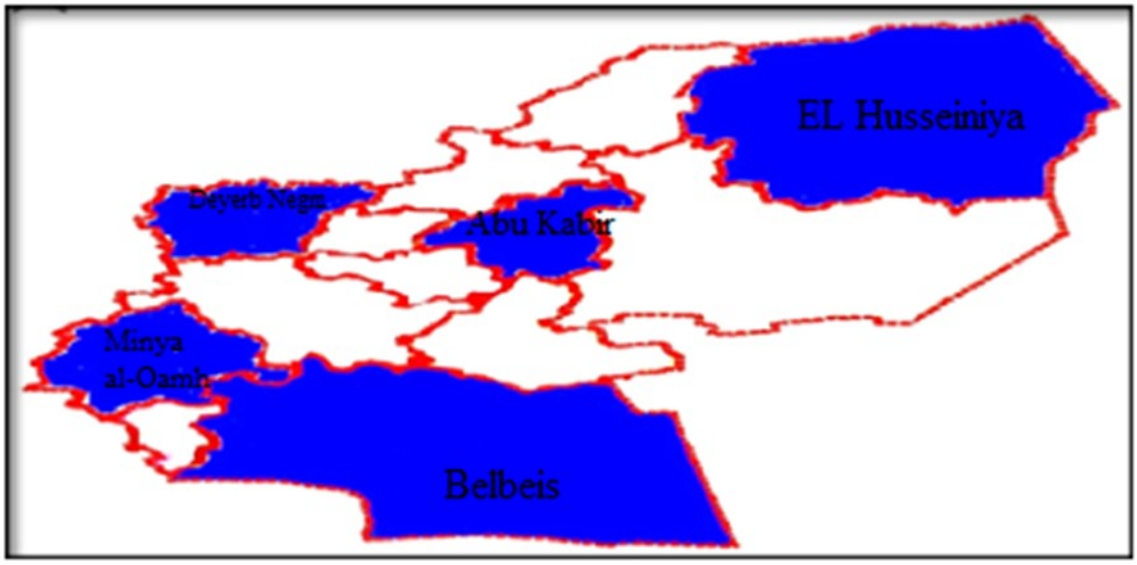

Figure1: The study area

2.2. Blood and Milk Sampling:

About 10 ml of blood was drawn from a jugular vein of the animal using a vacutainer tube. Tubes were incubated overnight at 4ºC and serum was then separated by centrifugation. The collected serum was labeled and stored at -20ºC until used for biochemical and serological determination. Raw milk samples (100 ml)were collected into sterile bottles with labels and kept in an ice box, transported to the laboratory for immediate bacteriological culturing and PCR.

2.3. Serological Examination:

Serum samples of examined animals were tested using mRBT as a screening test and complement fixation test (CFT) as a confirmatory test according to **Alton et al. (1988)** and **Blasco et al. (1994).**

2.4. Isolation and identification of Brucella species from milk samples:

Brucella species was isolated from milk samples of serologically positive she-camels according to **Alton et al. (1988)**. The milk samples have been centrifuged at 3000 rpm for 10 minutes for acquiring the sediment cream mixture. This mixture was used for culturing on Brucella medium base supplemented with 5% horse serum (Oxoid, CM 0169) and additionally on the Farrell′s selective medium. The inoculated plates

were incubated at 37ºC in the presence of 10% CO2 for two weeks. After incubation, the suspected colonies of *Brucella* were morphologically examined. *Brucella* colonies showed a round, glistening, pinpoint, and honey-drop-like appearance. The colonies had been examined for both Gram stain and Modified Ziehl-Nelsen stain. The biochemical identification and biotyping of *Brucella* isolates were once performed using oxidase, catalase, urease monospecific sera (A&M) and dyes sensitivity.

2.5. PCR Assay on milk for detection and identification of Brucella:

It was carried out as described by **(Bricker & Halling, 1994).**

Extraction of DNA from milk samples according to **leal-Klevezes et al. (1995).**

Oligonucleotide primers: Primers used were supplied from biobasic (Canada) and are listed in table (1).

PCR amplification: Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1 % agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. Generuler 100 bp DNA ladder (Fermentas, Thermo, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

2.6. Estimation of Oxidative Stress and Antioxidant Enzymes:

Assessment of lipid peroxidation: Malondaldehyde (MDA) concentration in serum of camels was used as the index of lipid peroxidation as described by **(Ohkawa et al. 1979).** MDA was determined by measuring the thiobarbturic acid reactive species. The absorbance of the resultant pink product was measured at 534 nm. Nitric oxide (NO), Glutathione Peroxidase enzyme (GPx), and Catalase (CAT) were calorimetrically determined using commercial kits (Biodiagnostics, Egypt).

2.7. Biostatistical Analysis:

Relative risk (RR= (a⁄a+b) / (c⁄c+d)) is a measure of the strength of the association or causal link between a risk factor and an outcome. Attributable risk (AR=(a⁄a+b)-(c⁄c+d)) helps determine the extra risk associated with the risk factor. Odds ratio (OR = ad / cb) measures the relative frequency of risk factors for brucellosis to occur in farms. Where: a = number of exposed seropositive, b = number of exposed seronegative, c = number of non-exposed seropositive and d = number of non-exposed seronegative, **(Hoffman, 2019).** Data on serum oxidative stress, and antioxidant parameters were subjected to t-test.

Table 1. Primers sequences, target gene and cycling conditions PCR.

Target gene	Target agent	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles) Secondary denaturation		Extension	Final extension	References
IS711	*B. abortus*	1S711-specificPrimer TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT B. abortus-specific Primer GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC	498	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Bricker and Halling, 1994
	B. melitensis	1S711-specificPrimer TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT B. melitensis-specific Primer AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA	731	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
	B. suis	1S711-specificPrimer TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT B. suis-specific Primer GCGCGGTTTTCT GAAGGTTCAGG	285	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	

3. RESULTS:

Table 2. Results of serological tests of dairy camels from different localities

District	No.	Serological tests	
		mRBT	CFT
Abu Kabir	20	2 (10%)	2 (10%)
Belbeis	85	15(17.6%)	12 (14.12%)
Deyerb Negm	20	1 (5%)	1 (5%)
EL Husseiniya	70	10 (14.3%)	9 (12.9%)
Minya al-Qamh	75	12 (16%)	8 (10.7%)
Total	270	40 (14.8%)	32 (11.9 %)

Revealed results of serological immunoassays of dairy camels-from different areas using modified RBT and CFT

Table 3. Distribution of brucellosis seropositivity and related risk variables in examined camels.

Risk factor	Animal status	Animal No.	Serological test			
			mRBT		CFT	
			+ve	%	+ve	%
Addition of new animals	Yes	150	29	19.3	26	17.3
	No	120	11	9.2	6	5
Mixed population	Yes	170	34	20	30	17.6
	No	100	6	6	2	2
Sanitary measures	Yes	112	12	10.7	8	7.1
	No	158	28	17.7	24	15.2

Showed brucellosis seropositivity and related risk variables, addation of new animals, mixed population and sanitary measures

Table 4. Magnitude of the associations between different risk factors and seropositivity of brucellosis with CFT in examined camels

Risk factor	Relative risk	Attributable risk	Odds ratio
Addition of new animals	3.5	0.12	3.98
Mixed population	8.8	0.16	10.5
Sanitary measures	0.47	-0.08	0.43

Reported the association between risk factors and brucellosis CFT seropositive in examined camels

Table 5. Results of bacteriological isolation and PCR assay of milk samples from serologically positive dairy camels.

Test performed	No. of animals	Positive		Negative	
		NO.	%	NO.	%
Bacteriological culture	32	3 (2)*B.melitensis biovar3* (1) *B.abortus biovar1*	9.4	29	90.6
PCR assay	32	22	68.8	10	31.2

isolation of (32) Brucella strains identified as 2 *B. melitensies biovar 3* and 1 *strain B. abortus biovar 1*. PCR Assay detect (22) Brucella species

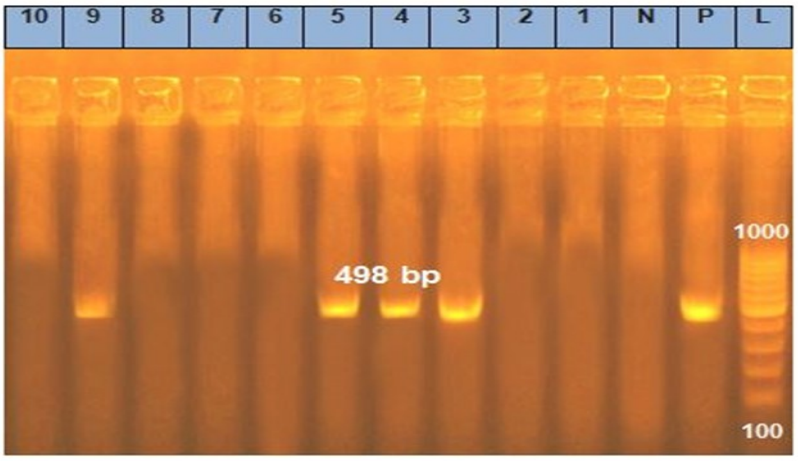

Figure (2). Amplified PCR products of *Brucella abortus*strain from camels. Agarose gel electrophoresis of PCR products. Lane L: Marker, Lane 3,4,5 and 9: *Brucella abortus*498 bp, negative and positive (*Br. abortus* 498bp) controls were included.

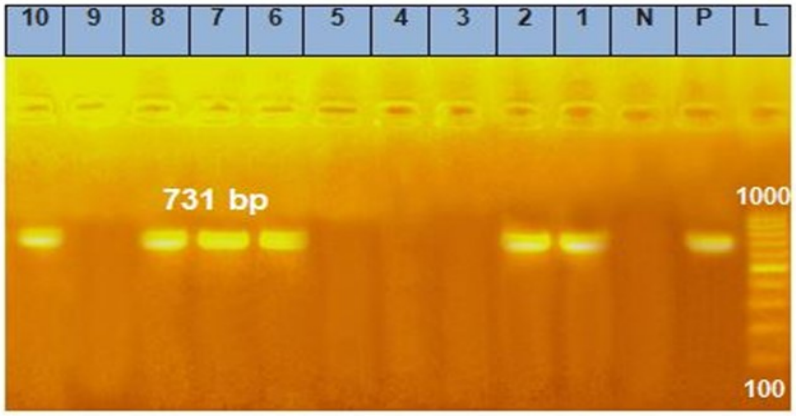

Figure (3). Amplified PCR products of *Brucella melitensis* from camels. Agarose gel electrophoresis of PCR products. Lane L: Marker, Lane 1,2,6,7,8and 10: *Brucella melitensis*731 bp, negative and positive (*Brucella melitensis*731 bp) controls were included.

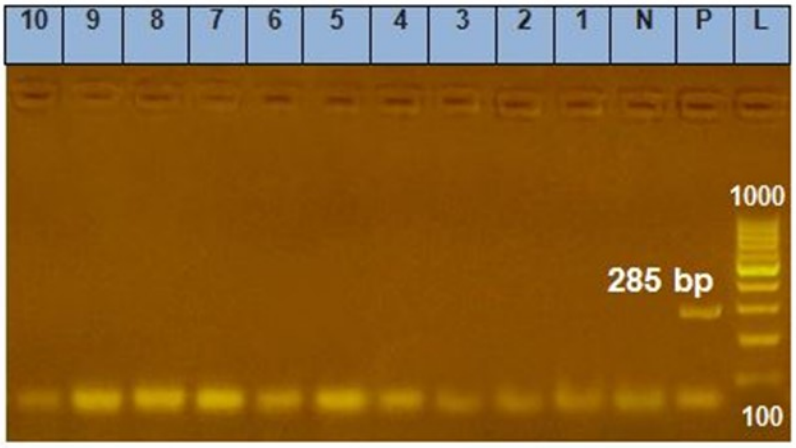

Figure (4). Amplified PCR products of *Brucella suis* from camels. Agarose gel electrophoresis of PCR products. Lane L: Marker, negative and positive (*Brucella suis*285 bp) controls were included.

Table 6. Oxidative stress and antioxidant biomarkers of Sero-negative and Sero-positive brucellosis in camel.

Parameters	Sero negative control	Seropositive infected Brucella
Oxidative stress biomarkers:		
MDA (n mol/ml)	0.74 ± 0.21	0.88 ± 0.71 **
NO (μ mol/ml)	0.49 ± 0.41	0.51 ± 0.78
Antioxidant biomarkers:		
GPx (u/l)	941 ± 223	1054 ± 176 **
CAT (u/l)	309 ± 20	270 ± 121 **

(MDA); Malonaldehyde, NO; Nitric Oxide, GPx; Glutathione peroxidase,CAT; Catalase, (mean ± SD); p value; ** Significantly ($P \leq 0.05$).

Oxidative Stress Parameters and Antioxidant Enzymes:

The results of different oxidative stress parameters and antioxidant enzymes in serum of brucellosis-infected and healthy camel are presented in Table 6. The results on different oxidative stress parameters recorded in brucellosis-infected camel indicated a substantial increase ($P \leq 0.05$) in values of lipid peroxidation product (MDA) while, nitric oxide in brucellosis-positive camel was moderate as compared to healthy animals. The results on different antioxidant enzymes showed significantly ($P \leq 0.05$) high values of GPxand lower values of CAT enzymes in infected camels as compared to healthy one.

4. DISCUSSION:

Brucellosis in animals causes great monetary losses due to premature birth, decreased milk production, reduced fertility and cross-transmission to other animal species. Modified Rose Bengal Test and Complement Fixation Test table (2) revealed a prevalence of 40 (14.8%) and 32 (11.9%) respectively. The three districts with the highest prevalence of camel brucellosis were Belbeis (14.12%), EL Husseiniya (12.19%), and Minya al-Qamh (10.7%). The greater responses were recorded with modified RBT 40 (14.8%). This result comes in accordance with **Hamdy et al. (2017),** who reported that mRB is the most suitable test that may be very beneficial as a screening test for testing camels for brucellosis. According to **Hosein et al. (2017),** buffered Brucella antigen tests offer a better sensitivity but a less reliable specificity, leading to a greater proportion of false positives compared to false negatives. In this study, it was evident that Complement Fixation Test gave lower reactors compared with mRBT 32 (11.9%) reactors table (2). Since the CFT exclusively detects IgG specific for brucella infection, it overcomes cross-reaction with other similar gram-negative bacteria and so, no false results are detected, therefore, the test is considered the gold standard serological test for diagnosing brucellosis **(Abernethy et al. 2012).**

The prevalence of brucellosis in dairy camel herds that introduced new animals of unknown status to household camels was 17.3% %, as illustrated in Table (3).Regarding the results summarized in Table (4), the relative risk, attributable risk, and odds ratio were 3.5%, 0.12%, and 3.98%respectively. These findings support the suggestion of **Earhart et al. (2009)** who claimed that the addition of new animals may increase the risk of brucellosis in herds and flocks. Importantly, the practice of purchasing animals raises the risk of introducing an infected animal into a herd which is linked to an increase in animal move-

ment both on the farm and off it. The main risk factors associated with animal brucellosis include the introduction of diseased animals into a susceptible herd, the unrestricted movement of animals, especially in markets, and the breeding of mixed-species animals **(Hosein et al. 2018)**.

Seroprevalence was higher (17.6%) in camels reared in mixed groups with other ruminants (cattle, sheep, and goats) compared with those camels raised alone (2%), as shown in Table (3). Values for the relative risk, attributable risk and Odds ratio table (4) were 8.8, 0.16 and 10.5, respectively. Our findings corroborate those of **Fatima et al. (2016),** who demonstrated a substantial correlation between the rearing of other ruminants and the occurrence of camel brucellosis. In addition, mixed husbandry practices have been linked to camel herds with brucellosis seropositivity, according to **Ghanem et al., (2009).** Moreover, **Hadush et al. (2013)** found that camel herds in close proximity to small ruminant animals were 3.6 and 2.3 times more probable to be seropositive for brucellosis. Such inter-species transmission situation may be the outcome of close contact between small and large ruminants and camels especially in developing countries when farm animal species are kept on the same premises without biosecurity precautions.

Table (4) showed that the lack of sanitary measures was an important risk factor, with a relative risk of 0.47 (a negative relationship) and an attributable risk of -0.08, indicating a link between sanitary precautions and seropositivity to Brucellosis. These outcomes are consistent with **AL-Majali (2008),** who found that lack of disinfection procedures is an important risk factor for Brucella seropositivity. A good Brucella control program must ensure that waste materials are disposed of properly and that all procedures are strictly sanitary. Results illustrated in Table (4) showed that there was a correlation concerning the associated different risk factors, (addition of new animals, multiple raising of animal species, and lack of sanitary measures) and the seropositive reactors of brucellosis which all agreed with **Radostits et al. (2007).** The isolation of specific etiological agents is required to establish a confirmatory diagnosis. The isolation and identification of Brucella Spp. are helpful for epidemiological studies and provide a definitive diagnosis of brucellosis. Brucella isolation is the gold standard for diagnosis but it takes a long time, is potentially hazardous, and needs highly qualified workers **(Wareth et al. 2014).**

In this study, bacteriological examination showed that *Brucella* microorganisms were isolated from three(9.4%) out of 32 milk samples from CFT serologically positive she-camels (table 5). *Brucella* isolation needs a high concentration of live bacteria in clinical samples, which may explain the low percentage of isolated Brucella organisms from camel's milk in this study. In addition, excretion of the pathogen through milk is intermittent **(Seleem et al. 2010).** Isolated strains were recognized as two isolates identified as *B. melitensis* biovar 3 and one isolate identified as *B. abortus*. *Brucella melitensis* primarily affects sheep and goats. Currently in Egypt, *Brucella melitensis biovar 3* is the prevalent field strain. Infection of camels with this biovar may be the result of close contact between (sheep, goats, and cattle) and camels. Camels are not recognized as the principal host for any Brucella species, although they can still be susceptible to both *B. abortus* and *B. melitensis* infections **(Musa and Shigidi, 2001).**

Molecular detection based on PCR is a promising option for the conclusion of infectious diseases caused by fastidious microorganisms such as *Brucellae*. *Brucella* strain DNA extracts from milk samples showed a specific amplicon of 731 bp specific for *B. melitensis* (18) and amplicon of 498 bp specific for *B. abortus* (4), but no amplicon of 285 bp specific for *B. suis* could be detected after being subjected to PCR using the IS711 primer in this investigation figures (2, 3 , 4). Such finding is consistent with that of **Hosny et al. (2017),** who stated that *Brucella meliteneis* is the most common strain found in camels, possibly as a result of interaction between camels and small ruminants. The obtained results agree with those obtained by **Hosein et al. (2016)** who stated that Egypt has a high rate of camel brucellosis. *Brucella melitensis* biovar 3, which is common in both large and small

ruminants, cattle and sheep in Egypt, constitutes the main source of the infection to camels raised in close proximity to these animals. The molecular findings obtained in this study are consistent with those of **Waleed et al. (2013),** who said that PCR assays revealed the capacity of PCR testing for the identification of *Brucella* from clinical samples that may be successfully employed in routine diagnosis of brucellosis. The current findings demonstrated that *Brucella* species may be promptly molecularly detected from clinical samples such as milk without the exhaustive efforts of isolating the pathogen first.

According to the aforementioned findings, PCR test has been demonstrated to be a useful technique for locating DNA of various *Brucella* organisms and offers a skilled preference in order to identify brucellosis. Moreover, when a culture failed or serological results were ambiguous, PCR proved to be a useful diagnostic technique. In addition, PCR has several benefits since it is more rapid and more responsive than conventional culture methods. It is feasible to reduce the chance that a laboratory worker would get the disease (**Queipo-Ortuño et al. 2005).**

Our findings conflict with those of **Aman et al. (2020),** who reported that out of 32 camels, the most prevalent *Brucella* strain, was *B. abortus*, found in 25 camels, followed by *B. suis* in 5, and *B. melitensis* in just 2 camels.

Our study observed MDA levels in animals infected with brucellosis were significantly higher than the control group (Table 6), this may be due to evaluate lipid peroxidation induced by free radicals acting upon membrane lipids. Oxyradical-induced cytotoxicity gives rise to lipid peroxidation through the reaction of free radicals and peroxides with fats in cellular membranes, resulting in malondialdehyde formation. infected Camel recorded significantly higher circulating malondialdehyde concentrations than did control groups. The excess serum malondialdehyde was produced by the inflamed intestine and translocated into the circulation. Our results agree with **Kandemir et al. (2002)** where indicated MDA levels were high in Brucellosis cases and that they dropped down to normal levels during recuperation after the treatment. Also, **McCord, (2000)** reported that increased free radical production and malondialdehyde (MDA) due to lipid peroxidation react with biological structures such as proteins, lipids, carbohydrates and DNA and cause damage**.** Lipid peroxidation is also responsible for tissue damage which causes ailments such as cancer, aging inflammatory diseases and atherosclerosis **(Inal et al. 2001).** Many researchers have reported that numerous pathogens suppress the immune system by promoting free radical production and causing tissue damage **(Burgner et al. 1999, Akaike and Maeda 2000** and **Pfister et al. 2002).**

Nitric oxide (NO) as a gas and a free radical is synthesized enzymatically from the amino acid L-arginine in a number of tissues using the three isoforms of nitric oxide synthase and the endothelium, is responsible for the regulation of blood flow and the activation of blood platelets **(Bruckdorfer, 2005).** In the present study, serum NO levels of the infected group have been found a moderate increase than the control group ($p \leq 0.05$). The moderate increase of NO in camels can be attributed to the decrease in lowered feed intake due to Brucella which results in the lowered synthesis of proteins or that endothelial NO as a potent vaso-relaxant is used to cause vasodilatations during inflammation, a consequence of either damaged/ dysfunctional endothelium and the downregulation of IL-1 β which induce nitric oxide synthase (NOS) and NO synthesis **(Rosselli et al. 1998)** and **(Orozco-Hernandez et al. 2003).** This decrease suggests that it may be a result of the response of phagocytic cells to infection. This finding was in agreement with previous data on animal or human brucellosis **(Wang et al. 2001).** Oxidativepathway has an important role in the destruction of intracellular bacteria by macrophages and polymorphonuclear leukocytes **(Serefhanoglu et al. 2009).** Only IL-6, IL-8 and IL-2R are elevated in brucellosis and the extent of elevation depends on the severity and clinical pattern of the disease. Moderate elevation in serum NO was comparable to that observed in previous studies. This explains the

absence or very rare occurrence of septic shock in brucellosis. The participation of the inducible isoform of NO synthase (iNOS) was confirmed by the finding of an increased expression of both iNOS mRNA and iNOS protein. These observations might help to explain (i) the acute outcome of *Brucella* infection in rodents, (ii) the low frequency of septic shock in human brucellosis and (iii) the prolonged intracellular survival of *Brucella*in humans.NO subject to a number of reactions in some biological conditions, which tend to involve the formation of both nitrate and nitrite ions. An increase in Nitrate level was a sign of NO in some infections such as in brucellosis (**Kandemir et al. 2002).**

Also, this study indicated a significant increase in the level of glutathione peroxidase (GPx) in the sera of camel infected with brucella compared with non-infected as shown in table (6). The presence of malondialdehyde in the circulation may explain the increased formation of glutathione as a means of preventing oxidative damage.In *Brucella* infected camel, serum glutathione peroxidase activities increased significantly which generally occurs in the oxidative stress to scavenge the free radicals and oxidative stress in the infected animals **(Kataria et al. 2010).** The antioxidant mechanisms of the body combat the oxidative stress induced by bacteria or decrease the availability of iron to bacteria via sequestering iron in mononuclear phagocytes, inducing bacterial growth inhibition **(Auer et al. 1989)** which is the first line of defense against oxidative stress induced by endogenous and/or exogenous factors and thus helps in maintaining the cellular integrity the increase of its activity is related to many diseases **(Ataya et al. 2012),** the activity of the enzymatic antioxidants increased to overcome the oxidative stress and induce protection **(Kumar et al. 2017).** The antioxidant effect is associated with an increase in the concentration of zinc, high cytokine and peroxidation levels **(Kocyigit et al. 2002).**

In the present study, CAT was significantly reduced ($P \leq 0.05$) in infected camels with *Brucella.* CAT is removed for the inhibition of OH- production that leads to the formation of hydrogen peroxide in the cells. The hydrogen peroxide formed by SOD is degraded by the action of CAT and it can cross the nuclear membrane and induce damage through enzymatic reactions **(Birben et al. 2012).** SOD, CAT, and glutathione peroxidase are vital antioxidant enzymes for intracellular protection **(Akram et al. 2021 and Miao, 2022).**

The *Brucella* existence depends on the concentration of CAT and SOD in the *Brucella*. Thus, SOD and CAT are vital in the removal of nitrogen radicals and free oxygen produced by the Brucella, while glutathione peroxidase (GPx) handles the diminution hydroperoxides intracellularly **(Landisand Tower, 2005).** The hydrogen peroxide generated during the process is deactivated by CAT. The CAT action is primarily limited to the Brucella's periplasm **(Sha et al. 1994)** and is responsible to supply safety against H_2O_2 produced during the immune reaction provoked against brucellosis. Control of CAT is necessary for the adjustment procedure of *Brucella* to endure and preserve under frightening circumstances. The biomarkers of oxidative stress in brucellosis-positive camel were significantly increased in the present study. The increased values of these biomarkers are suggestive of the induction of oxidative stress and indicate that the increased process of oxidation in erythrocytes is responsible for the rapid generation of free radicals, ultimately leading to inefficient antioxidant capacity and breakdown of erythrocytes **(Kumar *et al*. 2017 and 2020).** In addition, oxidative enzymes including MDA, ceruloplasmin, NO and Cu increased due to Brucellosis low concentration of antioxidant enzymes **(Akram et al. 2021).** Thus, increased oxidative stress causes breakage of DNA, lipid peroxidation, and protein denaturation **(Karsen *et al*. 2019; Birben *et al*. 2012 & 2017).**

5. CONCLUSION:

It is concluded that *Brucella meliteneis biovar* 3 is the common field strain detected from camels. Brucellosis-positive camels had considerably higher levels of the oxida-

tive stress biomarker MDA. At the time, the antioxidant enzyme (GPx) was increased markedly; these indicated its parallel reaction. This study has improved our understanding of the risk factors for camel brucellosis which are: (i) the rearing of camels with different ruminant species. (ii) the addition of new animals. (iii) the lack of sanitary measures, which are the primary risk factors for the introduction and spread of Brucellosis among dairy camels.

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