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Mycoplasma haemolamae infection in Camelus dromedarius in Egypt.

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This study was conducted on (82) collected blood samples from camels which obtained from abattoirs of Cairo governorate to study the presence of *Mycoplasma Haemolamae* which revealed that 31 (37.8 %) camels were infected with *Mycoplasma haemolamae.* The blood samples were examined for hematological and serum biochemical alterations associated with infected camel and positive samples were confirmed by polymerase chain reaction.

Keywords: ***Mycoplasma Haemolamae*, Hematological alterations, Serum biochemical changes, Camel, PCR**

INTRODUCTION

Mycoplasma haemolamae is formerly known as Eperythrozoonosis in camelids (Susan et al., 2010). *Mycoplasma haemolamae* showed similarities to other Mycoplasma spp. including *Mycoplasma wenyonii, Mycoplasma suis* and *Mycoplasma haemofelis* according to DNA sequence analysis of the 16s small subunit ribosomal RNA gene so, it classified within the mycoplasma genus as haemotrophic mycoplasmas (haemoplasmas). (Messick et al., 2002) (Pentecost et al., 2012)

Mycoplasma haemolamae known with its tropism for the red blood cells of camelids, it is a small gram negative bacteria, without a cell wall (Foley and Pedersen, 2001), it bind to red blood cell membrane of infected animal and it's a sensitive bacteria to tetracycline (Hoelzle, 2008) (Messick, 2004). Generally appear as coccoid or ring-shaped organisms that are found adherent to the periphery of erythrocytes, or free in the background of the blood smear. (Susan et al., 2009)

Infection with *Mycoplasma haemolamae* has the capability to impact the camelid industry by generating chronically infected camels without clinical signs of disease with weight loss and inappetance (Susan et al., 2011). The obvious clinical signs of disease are generally observed in calves, stressed or immuno compromised animals and can include recumbency associated with haemolytic anaemia which can lead to animal death or a mild long standing anaemia, infertility and suppression of animal immunity. (Hoelzle, 2008) (Almy et al., 2006).

The mode of infection with *Mycoplasma haemolamae* in camelids is not clearly known, but blood sucking arthropods such as ticks are highly suspected (Almy et al., 2006). Experimentally, transfusion of infected blood with *Mycoplasma haemolamae* transmits this organism to the animal resulting in parasitemia. (Delgado and Timenetsky, 2001)

Mycoplasma haemolamae is widely spread all over the world and the prevalence rates with PCR based assays showing a range of 9.3-19.3% for camelids in South part of America and a prevalence of 18.6% of the overall population (Pentecost et al., 2012). Diagnosis was basically dependent on cytological evaluation of blood

smears with molecular (PCR) based testing for *Mycoplasma haemolamae* (Pentecost et al., 2012). These PCR assays overt accurate identification of infected animals regardless of the presence of clinical signs (Kaufmann et al., 2010).

Our study aimed to assess the hematological and biochemical alterations associated with *Mycoplasma Haemolamae* in naturally infected camels in Egypt, with subsequently confirmation using PCR technique

MATERIALS AND METHODS

Animals:

Eighty-two blood samples were collected from camels which obtained from abattoirs of Cairo governorate. We collect two types of blood samples from (Jugular vein). The first one collected in EDTA for hemogram evaluation and PCR assessment. The second one was collected in plain centrifuge tube for clot and serum separation for studding of clinico-biochemical alterations.

Hematological examinations:

Hematological studies including erythrogram (red blood cell count, hematocrit and hemoglobin measurement), while Leuckogram including total leucocyte count and differential leucocytic count (Feldman et al., 2000).

Biochemical examinations:

Serum biochemical evaluation for alterations in the level of glucose, serum total proteins and albumin (Dumas and Biggs, 1972), creatinine (Tabacco et al., 1979) and BUN concentrations (Fabiny and Ertingshausen, 1971), evaluation for changes occurs in some liver enzymatic activities as alanine amino transferase (ALT) (Reitman and Frankel, 1957), aspartate amino transferase (AST) (Tietz, 1986), alkaline phosphatase (ALP) (Dumas and Biggs 1972) with gamma glutamyl transferase (GGT) (Doumas et al., 1973) all tested with reagent kits supplied by StanBio Laboratories incorporation, USA.

Reference strain

The reference sample of *Mycoplasma haemolamae* was collected from cases of chronic anaemia in camels and identified by PCR in Microbiology Department, Faculty of Veterinary medicine, Cairo University.

PCR analysis

DNA extraction.

Qiagen DNA blood minikits (Qiagen) according to the manufacturer's instructions were used for DNA extraction from EDTA- blood samples. DNA concentration and degree of its purity was measured using absorbance ratio between 260/280 nm (Nanodrop, Thermo Scientific, USA).

PCR assays.

Extracted DNA from examined samples was tested for the presence of *Mycoplasma haemolamae*. 1000-bp fragment of the 16S rRNA gene was amplified using oligonucleotides 16s munivF (5'-AGA CTC CTA CGG GAG GCA GCA-3') and 16s munivR (5'-ACT AGC GAT TCC GAC TTC ATG-3') according to Alberto et al. (2006). Amplification was performed in a DNA thermalcycler-Perkin Elmer/Cetus Research, USA. For 16S rRNA gene PCR, the initial denaturation was performed at 94°C for 9 min, followed by 35 cycles at 94°C for 45 s, 60°C for 60 s, and 72°C for 90 s, with a final extension at 72°C for 5 min. The amplicons were electrophoresed in 2% agarose gel incorporated with ethidium bromide and visualized using a UV transilluminator.

Statistical analysis:

Mean and standard error of mean (Mean±SE) were determined. The difference in means was tested for significance by using Statistics by Student's t test according to Sendecor and Cochran (1982).

RESULTS

Table 1: Hematological parameters of camel *Mycoplasma haemolamae* negative and positive groups.

Hematological parameters	*Mycoplasma haemollama* negative	*Mycoplasma haemollama* positive
PCV(%)	34.66 ±2.18	26.37± 2.25*
Hb (g/dl)	10.33±2.02	8.72±1.64*
RBCs($\times10^6$/µl)	11.03 ±1.28	8.60 ±1.25*
MCV(Fl)	31.4 ± 3.08	29.59±2.10*
MCHC (g%)	39.45 ± 3.91	28.03 ±1.14*
TLC($\times10^3$/µl)	9.86±1.05	13.95 ±1.07*
Neutrophil ($\times10^3$/µl)	7.25 ±3.43	9.03 ±2.34*
Lymphocyte ($\times10^3$/µl)	1.57 ± 0.24	3.42 ± 0.15*
Monocyte ($\times10^3$/µl)	0.60 ±0. 04	0.76 ±0. 08
Eosinophil ($\times10^3$/µl)	0.43 ±0.03	0.52 ±0.06

* represents significantly different between *Mycoplasma haemolamae* negative and positive groups at probability P< 0.05.

Table 2: Serum biochemical parameters of camel *Mycoplasma haemolamae* negative and positive groups.

Biochemical parameters	*Mycoplasma haemolama* negative	*Mycoplasma haemolama* positive
Glucose (mg/dl)	76.25 ± 6.25	39.58 ± 4.15*
T.protein (g/dl)	9.56 ± 1.15	6.28 ± 2.13*
Albumin(g/dl)	4.54 ±0.30	2.34 ± 0.53*
globulins(g/dl)	4.62 ± 0.56	3.95 ± 1.08
A/G	0.93 ± 0.32	0.59 ± 0.11*
AST(U/L)	80.28 ± 23.71	79.7 ± 21.43
ALT(U/L)	23.1 ± 4.57	22.82 ± 3.71
ALP(U/L)	49.95 ± 11.14	47.89 ± 15.02
GGT(U/L)	24.36 ± 5.38	25.46 ± 6.54
BUN(mg/dl)	46.58 ± 8.11	49.21 ± 6.97
Creatinine(mg/dl)	1.72 ± 0.32	1.58 ± 0.26

* represents significantly different between *Mycoplasma Haemolamae* negative and positive groups at probability P< 0.05.

Hematology

Examination of 82 camels using Giemsa-stained blood smears revealed that 31 (37.8 %) camels were infected with *Mycoplasma haemolamae.*

Numerous small, epicellular and coccoid to ring shaped structures consistent in appearance with *Mycoplasma haemolamae* were present on the surface of infected red blood cells or freely in the background single or in clusters (Fig 1, 2).

Mean values of the hemogram of camel groups are illustrated in table (1). Comparing the mean values of *Mycoplasma haemolamae* positive group of camels with those values of *Mycoplasma haemolamae* negative group, PCV, Hb concentration, RBCs count and red blood cell indices (MCV and MCHC) values showed significant decreases. Microscopical examination of the stained blood film showed macrocytosis, poikilocytosis, anisocytosis (Fig.3) with nucleated erythrocytes in positive group. Additionally, leucocytosis, neutrophilia with lymphocytosis were noticed in *Mycoplasma haemolama* positive group.

PCR detection of Mycoplasma spp

Of 31 hematologically positive samples collected from camels 100% were positive for the presence of 16S rRNA gene of *Mycoplasma* spp. (Fig. 4a &b)

Results of different biochemical parameters:

Statistical analysis of different biochemical parameters in serum of camel groups are illustrated in table (2). Comparing the mean values of *Mycoplasma haemolamae* positive group with those values of *Mycoplasma haemolamae* negative group exhibited significant hypoglycemia, hypoproteinemia, hypoalbuminemia with decrease in A/G ratio and insignificant changes in both hepatic biomarkers as well as renal biomarkers.

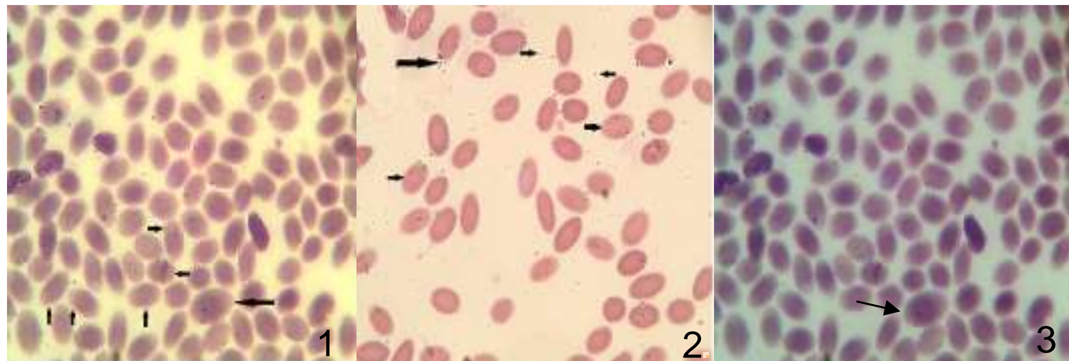

Figure 1 and 2; Light micrograph of Mycoplasma haemollama found individually and in chains surrounding and on the surface of red cells. Note the rod-shaped, spherical, and ring shaped forms. Figure 3; Light micrograph of Mycoplasma haemollama showed macrocytosis (black arrow), poikilocytosis and anisocytosis

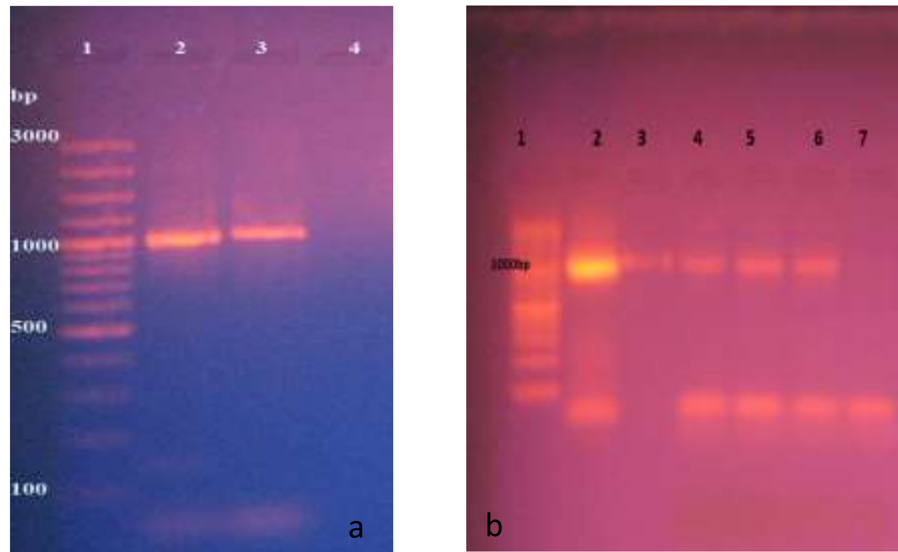

Figure 4 ;Gel electrophoresis of Mycoplasma spp. 16S rRNA PCR

Lane 1(a& b): Size marker (in base pairs, bp).lane 2(a), Lanes 3-6(b) positive samples. Lanes 3(a) and 2 (b) positive controls. Lanes 4(a) and 7(b) negative controls

DISCUSSION:

Hemoplasmas silently infecting animals and lead to disease only in stressed conditions (Messick, 2004). In our study, thirty one (37.8%) from eighty two examined camels was found infected with *Mycoplasma haemolamae.* Most of the positive animals appear to be subclinically infected without any clinical signs. The infection turned to clinical as the infected animal lies under any stress factors or as result of secondary infection (Crosse et al., 2012). In recent researches the infection rate was 29 % in alpacas in England (Crosse et al. 2012), 18.4 % in Peruvian alpacas and llamas, 9.26 % in Chilean alpacas (Susan et al. 2010) with 12 % reported in llamas in the USA (McLaughlin et al., 1990).

Diagnosis of Hemoplasmas was firstly depending on microscopic examination of stained blood film during acute phase of infection with marked bacteremia (almost all of red blood cells being infected). However, the sensitivity of a blood film examination is mostly less than 20% in cases of chronic infection, stain precipitates and Howell-Jolly bodies in examined blood film effect on the specificity of a blood smear examination (Maggi et al., 2013) but in our study all cytologically positive samples are PCR positive with 100%.

Hemotropic mycoplasmas are the causative agents of infectious anemia in many vertebrate animals worldwide (Willi et al., 2006), by colonise and replicate on red blood cells which may decreased life span of erythrocytes causing severe anemia in acute infected animals (Ana et al., 2012), or due to changes occurred in erythrocyte that stimulate the host immune response leading to hemolysis of infected cells (Lascola et al., 2009). Hematological parameters showed microcytic hypochromic mild regenerative anemia in *Mycoplasma haemolamae* positive group as *Mycoplasma haemolamae* considered as a nutrient scavenger that parasitize the erythrocytes of the infected camels that can lead to haemolysis of RBC's secondary to colonization and replication of *Mycoplasma haemolamae* on RBC's(Crosse et al., 2012).

Bacteraemia with *Mycoplasma haemolamae* was identified by cytological evaluation of blood smears of infected animals and observed as numerous coccoid and ring shaped, epicellular, basophilic organisms that attach to the surface of host erythrocytes and freely in the background. Significant leukocytosis with lymphocytosis was observed in infected camels which may be attributed to ability of mycoplasmas to establish chronic infections and produce super antigens, which bind directly to major histocompatibility complex molecules which stimulate large numbers of lymphocytes (Cole and Alkins, 1991, Messick, 2004).

Infected camels showed significant hypoglycemia, hypoproteinemia as well as hypoalbuminemia. Hypoglycaemia may be result when glucose utilization by mycoplasma is greeter

than gluconeogenesis by infected animal (Almy et al. 2006), or due to exaggeration for the glycolytic activity of infected erythrocyte (Sutton, 1977). Hypoglycemia usually come in parallel to Mycoplasma infection in many species including camelids (McLaughlin et al., 1990) and lambs (Burkhard and Garry, 2004).

Moreover, hypoproteinemia is common finding in diseased camels especially with long standing disease process and decrease in albumin level is even more common due to the relative lack of alpha globulin, so decrease in one usually means decrease in the other (Cebra et al., 2014).

CONCLUSION

From the present study, it is concluded that mycoplasma is clearly noticed though cytological examination of infected blood film but it's in need for more advanced techniques, as PCR amplification and sequencing of DNA, which facilitated the diagnosis for mycoplasma haemolamae infection.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All the authors significantly contributed to compile and revise this manuscript. AMT designed and performed the study, HMG reviewed the literature, critically revise the manuscript and check the English language accuracy. Finally all authors read and approve the manuscript for publication.

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