

## SEROCLINICAL DIAGNOSIS OF *Anaplasma marginale* BACTERIA IN CARRIER ARABIAN ONE - HUMPED CAMELS

Hasanain AJ. Al-Gharban\* ; Hala SR. AL-Tae\*\*

\*Department of Internal and Preventive medicine, College of Veterinary Medicine,  
University of Wasit, Wasit, Iraq

\*\*Department of Microbiology, College of Veterinary Medicine, University of Wasit,  
Wasit, Iraq

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### ABSTRACT

Anaplasmosis is a tick-borne disease of ruminants and wild animals that caused by an intra erythrocytic bacterium, *Anaplasma marginale*. Under natural conditions, camels become infected in areas where the disease is endemic. Camels that survive from acute infection become carriers because of the capability of these bacteria to deception the immune system using antigenic variations. Although, several serological methods were concerned for *Anaplasma marginale* IgG antibodies detection, but the competitive indirect ELISA test was more sensitivity and specificity. The present study was conducted at Al-Najaf and Wasit provinces on 120 camels, selected randomly from both sexes and divided into two aged groups. The total sero positivity prevalence was (10.83%); and depending on provincial basis was (8.57%) in Al-Najaf and (14%) in Wasit provinces. Clinically, the sero positive prevalence two age groups (<5 and >5 years old) had (6.67%) and (15%), respectively. No significant differences ( $P < 0.005$ ) were encountered in sero positive camels in related to sex and vital signs (temperature, respiratory and heart rates), emaciation and paleness of the mucous membrane, while the rough hair coat and presence of ticks encountered a significant difference ( $P < 0.05$ ).

### INTRODUCTION

The dromedary camel (*Camelus dromedarius*) is the most important livestock populations of numerous countries in the desert and semi-desert areas of Asia, Northern and Eastern Africa, South America as well as the high mountains of the Andes (1, 2). In

Iraq, about 65,000 of camels are found, all one humped (*Camelus dromedarius*), according to the FAO statistics of 2014 (3). All Bedouin groups and communities in diverse ecozones throughout Iraq are depending on camels for their livelihood. This reliance consists of utilization of camel milk, meat, and leather and wool. In addition, camels have used as animals for packing, transport and riding (1).

In their natural desert habitat, where camels are usually raised particularly during the long dry season, camels are subjected to severe stress conditions which render them susceptible to many diseases and ailments (4). In the past, for a fairly long time and due to scarce of studies about camel diseases, the scientists were consider the camels resistant to many disease causing factors (5). It has been a proved that camels are susceptible, the same as other livestock or even more, to the common disease causing pathogens affecting other animal species (6, 7). Usually, the camel's diseases are, often, difficult to deal with and having a very similar with non-specific signs, specific pathogens; suffer from common diseases of ruminants and are resistant to some pathogens (8, 9).

Anaplasmosis is one of the infectious non-contagious disease that caused by an obligate intraerythrocytic bacteria, *Anaplasma marginale*, belonging to the family Anaplasmataceae, order Rickettsiales. All members of the family Anaplasmataceae are obligate intracellular bacteria that replicate while enclosed in a eukaryotic host cell membrane-derived vacuole (10, 11). The disease is worldwide distribution, particularly, in tropical and sub tropical areas that afflicted a domestic and wild animals (12). Anaplasmosis is transmitted biologically by ticks, and mechanically by flies and contaminated fomites (13). Following transmission, *A. marginale* invades and multiplies within mature erythrocytes, only, resulting in an acute stage of disease that characterized by fever, progressive anemia, jaundice, digestive disturbances and emaciation (12, 14). The recovering from acute stage resulting in a persistent (carrier) animal that serves as long-term reservoirs to transmission the infection for susceptibles within a herd (12, 15).

The diagnosis of infection may be made, tentatively, based on geographic location, season and presenting clinical signs and/or necropsy findings observed in infected animals (16). In order to confirm the diagnosis, the laboratory techniques such as the microscopic evaluation of stained blood smears or serological / molecular diagnostic procedures are required. The last procedures are the only means for identifying the persistently infected,

subclinical carrier cattle. Producers in endemic areas often suspected in based on a history of the previous disease outbreaks in that locality (11, 17). ELISA has been used for diagnosis of *A. marginale* infection in various ruminants including cattle, buffalo, sheep and camel (18, 19, 20, 21). The competitive indirect ELISA, based on recombinant MSP5 for *A. marginale* antibody detection, was developed by (22), which commercially available by Svanova Biotech AB (Uppsala, Sweden) and appear to be the test of choice for screening the carrier cases because of the high estimated sensitivity, rapidity in obtaining results, relative low cost and ease of standardization (16, 22). The objectives of study were:

1. Determine the seroprevalence of carrier camels with IgG antibody against *A. marginale* infection, for first time in Iraq.
2. Investigate the clinical case history for seropositivite camels.
3. Provide a baseline data about carrier anaplasmosis for first in Iraq.

## MATERIAL AND METHODS

### 1. Regions, case history and blood sample

The study was conducted from January-August / 2015, involved 120 one-humped camels (70 from Al-Najaf and 50 from Wasit provinces / Iraq), selected randomly of both sexes and aged from (1-10) years old. Camels submitted to the clinical examination that involved the area, age, sex, body temperature, heart and respiratory rates, rough hair coat with emaciation, decreasing in milk production, abortions status, paleness of mucous membrane and presence or absence of ticks. (3ml) of blood were drained from each animal by jugular vein-puncture under aseptic condition by using a disposable syringe and installed in tubes (without anticoagulant) for serological diagnosis. After collection, samples were transported to the laboratory, centrifuged, and frozen under -20°C in 1 ml micro-tubes.

### 2. Serological test

The test was performed by using a commercially available *A. marginale* competitive indirect ELISA, (Svanova Biotech AB, Sweden) kit.

### 3. Statistical analysis

All data was analyzed by a computerized IBM SPSS (v.23) programme. Chi-square ( $\chi^2$ ) and t-test were used to determine the significant differences between the study area, age,

sex and the clinical case history groups, with seroprevalence of infection. The differences were be considered statistically significant at ( $P<0.05$ ) (23).

## RESULTS

Out of 120 examined camels, the total seropositive prevalence was 13/120 (10.83%), detailed as 6/70 (8.57%) and 7/50 (14%) in AL -Najaf and Wasit provinces / Iraq, respectively, table (1).

According to age, 4/60 (6.67%) and 9/60 (15%) of less and larger than 5 years, respectively, were seropositives. In related to sex, 1/10 (10%) male and 12/110 (10.9%) females were seropositives, table (2).

In table (3), the clinical examination's results of vital signs (temperature, respiratory rate and heart rate) were ( $39 \pm 0.4^{\circ}\text{C}$ ), ( $10 \pm 1$  per / min) and ( $46.4 \pm 0.5$  per/min.) in seropositive camels; while ( $38.5 \pm 0.2^{\circ}\text{C}$ ), ( $9.6 \pm 0.7$  per / min) and ( $44.3 \pm 0.2$  per/min) in seronegative camels, respectively. Although, the significant differences ( $P<0.05$ ) were encountered between study areas and age groups, it's not indicated in sex and vital signs groups.

In related to table (4), that investigated the correlations in clinical case history (emaciation, rough hair coat, decreasing of milk production, abortion status, paleness of mucous membrane and presence of ticks) between seropositive and seronegative camels, the results indicated significant differences ( $P< 0.05$ ) in rough hair coat, decreasing of milk production, abortion and presence of ticks.

**Table 1: Seroprevalence of carrier anaplasmosis according to regions**

Province (Regions)	Total Camels (n=120)	Seropositives	Seronegatives
Al-Najaf	70 (58.33%)	6 (8.57%) <sup>b</sup>	64 (91.43%)
Wasit	50 (41.67%)	7 (14%) <sup>a</sup>	43 (86%)
Total	120	13 (10.83%)	107 (89.17%)

The difference in small letters, vertically, refers to a significant difference at level  $P< 0.05$

**Table 2: Seroprevalence of disease according to age and sex**

Factors	Total Camels (n= 120)	Seropositivity	Seronegativity
<b>Age</b>			
< 5 year	60 (50%)	4 (6.67%) <sup>b</sup>	56 (93.33%)
> 5 years	60 (50%)	9 (15%) <sup>a</sup>	51 (85%)
<b>Sex</b>			
Male	10 (8.33%)	1 (10%) <sup>b</sup>	9 (90%)
Female	110 (91.67%)	12 (10.9%) <sup>b</sup>	98 (89.1%)

At the same group, difference in small letters, vertically, refers to a significant difference at a level  $P < 0.05$

**Table 3: Vital signs in Seropositive and Seronegative Camels**

Vital signs	Seropositives M ± SE	Seronegatives M ± SE
<b>Temperature / minute</b>	(39 ± 0.4) <sup>b</sup>	(38.5 ± 0.2) <sup>b</sup>
<b>Respiratory rate / minute</b>	(10 ± 1) <sup>b</sup>	(9.6 ± 0.7) <sup>b</sup>
<b>Heart rate / minute</b>	(46.4 ± 0.5) <sup>b</sup>	(44.3 ± 0.2) <sup>b</sup>

Difference in small letters, horizontally, refers to a significant difference at level  $P < 0.05$

**Table 4: Clinical case history in Seropositive and Seronegative Camels**

Clinical signs	No.	Seropositives No=13	Seronegatives No= 107
<b>Emaciation</b>	<b>37</b>	<b>4 (30.77%)<sup>b</sup></b>	<b>33 (30.84%)<sup>b</sup></b>
<b>Rough Hair Coat</b>	<b>33</b>	<b>5 (38.46%)<sup>a</sup></b>	<b>28 (26.17%)<sup>b</sup></b>
<b>Decreasing of Milk Production</b>	<b>15</b>	<b>1 (7.69%)<sup>b</sup></b>	<b>14 (13.1%)<sup>a</sup></b>
<b>Abortions Status</b>	<b>14</b>	<b>1 (7.69%)<sup>b</sup></b>	<b>13 (12.15%)<sup>a</sup></b>
<b>Paleness Mucous Membrane</b>	<b>20</b>	<b>2 (15.38%)<sup>b</sup></b>	<b>18 (16.82%)<sup>b</sup></b>
<b>Presence of Ticks</b>	<b>19</b>	<b>3 (23.1%)<sup>a</sup></b>	<b>16 (14.95%)<sup>b</sup></b>

Differences in small letters, horizontally, refers to  
a significant differences at level  $P < 0.05$

## DISCUSSION

Although, an importance of carrier animals in transmission of disease causing a major threat to livestock, a very little work has been achieved for carrier anaplasmosis in camels, worldwide. However, carriers are immune to re-infection, but act as reservoirs for transmission and this contribute, a markedly, to extent of disease. During persistent infection, the infected erythrocytes, usually, are not detectable in stained blood smears and the diagnosis is, always, made by using of serologic techniques to detect the specific IgG antibodies against *A. marginale* (24, 25, 26). (27) Reported that the competitive ELISA was positive in acutely infected with *A. marginale* before or during the development of rickettsaemia, and that the antibodies were detectable in sera from persistently infected animals. In this study, the results indicated that *A. marginale* were extremely widespread in the examined areas, especially in Wasit province. Under natural conditions, camels might be infected where the disease is endemic in cattle population and persistence of ticks in agro-ecological zones. The variation in seropositivity prevalence between study's areas may caused by numerous physical, biological and socioeconomic factors (such as topographical conditions, cattle distribution, vector infestation, temperature, moisture,

pasture disruption) that interact to influence on the nature and extent of animals agriculture practiced in any region (28, 29, 30, 31).

In this study, the association between the seropositivity and age was encountered, and reported that the seroprevalence of carrier anaplasmosis was increased in more than (5 years) group. (25 and 32) reported that the persistent infection with *Anaplasma marginale* was prevalent in all age groups and increased with increasing age. The newborn receive, from an immune mother, a temporary protection by the colostrum that prevents the disease occurrence, and this protection will lasts for about three months in most cases, and followed by an age resistance that lasts until the animals about (9-12) months of age. Animals over 2 years of age are, usually, affected by a per-acute form of disease, and the severity of illness as well as the percentage of deaths would increase with age (33, 34, 35). This might be attributed to fact that the age's resistance to disease was gradually wanes after one year of age and the animals become increasingly susceptible to the disease in the regions which have no endemic stability (36, 37).

Whilst several studies were dealt with the relationship of sex with acute anaplasmosis, not with carrier, and almost of them referred to that a significant increase of anaplasmosis were indicated in females other than males, and this attributed to a number of factors related to females such as milk production, hormonal disturbances and weakness of immune system during gestation (38, 49, 40). However, the result of this study reported that both seropositive sexes were not showed any significant differences, and this result might be either due to independence of this parameter or due to that both, males and females, were exposed to the same level of risk factors such as the ticks (41, 42).

(43) Reported that, during acute anaplasmosis, camels showed, clinically, a significant increase in body temperature, respiratory and heart rates. Also, he indicated a number of signs such as emaciation, loss of appetite, paleness of mucous membrane, lacrimation, rough hair coat and presence of ticks on different regions of body. (44 and 45) reported that, the recovered animals from acute anaplasmosis would remain carriers for the rest of their live but without showing any clinical signs, and presence of these animals either in their herds, or neighboring herds, would makes it act as an effective source for infection. In present study, the seropositive camels were not reported significant differences ( $P < 0.05$ )

in temperature, respiratory and heart rates, emaciation and paleness of the mucous membrane, while the rough hair coat and presence of ticks encountered a significant difference ( $P < 0.05$ ). However, the presence of internal and external parasitic infections had been reported to be a major problem that causes severe damage and affecting on the health, productivity, and performance of camels (46, 47, 48). Noticeably, the increased contact between camels and other farm animals and presence of ticks may explain the increasing camels' diseases (49, 50). Relatively little information is available on the role of ticks as disease vectors in camels. It has been suggested that heavy tick infestations in a camel herd contributed to reduced growth rates and higher calf mortality in comparison with other herds in Kenya where tick control programs were adopted (51, 52, 53).

### **CONCLUSION**

The study was the first one in Iraq that dealt with carrier anaplasmosis in camels, which revealed the high rate of infection, especially, in Wasit province / Iraq, with the effectiveness of competitive indirect ELISA in detection of these carriers. Also, the results, of clinical examination, were reported in seropositive camels.

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## التشخيص المصلي والسريري لجراثيم *Anaplasma marginale* في الجمال العربية ذات السنم الواحد الحاملة للاصابة

حسنين عبد الحسين جعفر\* ، حلا سعيد رشيد\*\*

\* فرع الطب الباطني والوقائي ، كلية الطب البيطري ، جامعة واسط ، واسط ، العراق

\*\* فرع الاحياء المجهرية ، كلية الطب البيطري ، جامعة واسط ، واسط ، العراق

### الخلاصة

داء الانابلازما هو مرض ينتقل بواسطة القراد يصيب المجترات والحيوانات البرية ، تسببه بكتريا *Anaplasma marginale* التي تصيب كريات الدم الحمراء . تحت الظروف الطبيعية ، تصبح الجمال مصابة في المناطق التي يستوطن فيها المرض . الجمال التي تبقى على قيد الحياة بعد الاصابة الحادة تصبح حاملة للمرض بسبب قابلية البكتريا على خداع الجهاز المناعي بواسطة تغيير مستضداتها الجينية . بالرغم من وجود عدة طرق مصلية لتشخيص الاجسام المناعية IgG لل *Anaplasma marginale* ، لكن اختبار الاليزا التنافسي الغير مباشر اكثر حساسية واكثر انتقائية . اقيمت الدراسة الحالية في محافظتي النجف وواسط وتضمنت (120) جملا تم اختيارها عشوائيا من كلا الجنسين وقسمت الى مجموعتين عمريتين . بلغت نسبة الاصابة الكلية (10.83%) ، كانت نسبة الاصابة في محافظة النجف (8.57%) وفي محافظة واسط (14%) . سريريا ، كانت نسبة الاصابة في المجموعتين العمريتين ( اقل من خمس سنوات واكثر من خمس سنوات ) (6.67%) و ( 15%) ، على التوالي . لم يلاحظ وجود فروقات معنوية في الجمال الموجبة للاصابة مصليا فيما يتعلق بالجنس والفعاليات الحيوية (درجة الحرارة ، معدلات التنفس ودقات القلب ) ، والهزال وشحوب الاغشية المخاطية ، بينما لوحظ وجود فرق معنوي ( $P<0.005$ ) في طبقة الشعر الخشن ووجود القراد .

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