INCIDENCE OF ANAPLASMOSIS AND FMD IN CAMEL (*Camelus dromedaries*)

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ABSTRACT

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A total of 138 whole blood and serum samples were collected from camels (Camelus dromedaries) aged 3~10 years, with suspected clinical symptoms of blood parasites from May to August 2011, at different middle regions of Al-Riyadh Province in the Kingdom of Saudi Arabia (Al-Riyadh, Thumamah, Dawadmi and Wadi Al-Dawasir). Clinically the suspected infected camels with anaplasmosis showed signs of pale mucous membranes, rough hair coat, lacrimation, and ticks were noticed and detected on several locations at the camel's body. A total of 32 samples (23.19%) were microscopically positive for *Anaplasma marginale* (with parasitaemia 3-5%). All samples were tested by an indirect Enzyme Linked Immunosobent Assay (3-ABC-ELISA) which is specific to detect antibodies of foot and mouth disease virus (FMDV) infection. ELISA results revealed that 19 (13.76%) serum samples were positive; all these samples were positive for *Anaplasma marginale*. Meanwhile, the animals not showed any clinical FMD symptoms. This study revealed moderate infection of *Anaplasma marginale*, as well as concurrent incidence of FMD among camels in KSA. It would be of great interest to know if dromedaries play a role in transmitting the FMDV.

***Key words**: Anaplasma marginale, Camel, FMD, immunosuppression, 3-AB, ELISA.*

إنتشار مرض الحمى القلاعية والانابلازما مرجينال في الجمال (وحيدة السنام)

رجب محمد الخطيب ، كمال صابر مظلوم ، حبيب مقبول النخلى

تم جمع ١٣٨ عينة من الدم الكامل والمصل من الجمال التي يتراوح أعمارها من ٣-١٠ سنوات والتي يظهر عليها إعراض سريريه لمرض الانابلازما وذلك من شهر مايو إلى شهر اغسطس٢٠١١ من المناطق الوسطى بمحافظة الرياض بالمملكة العربية السعودية (الرياض والثمامة والدوادمى ووادي الدواسر). الجمال محل الدراسة كانت عليها علامات شحوب الأغشية المخاطية وخشونة في الشعر ودمعان مع تواجد القراد على أماكن متفرقة من الجسم. أظهرت نتائج فحص الشرائح الدموية ان عدد ٣٢ عينة كانت ايجابية لوجود طفيل الانابلازما مرجينال بنسبة (٢٣,١٩%). وباستخدام تفاعل الإنزيم المناعي المرتبط الغير المباشرة (3ABC-ELISA) والدال علي سبق الإصابة بفيروس مرض الحمي القلاعية وجد أن ١٩ عينة مصل كانت ايجابية لوجود الأجسام المضادة لمرض الحمى القلاعية

بنسبة (١٣,٧٦%) كما أن هذه العينات كانت إيجابية للإصابة بطفيل الانابلازما مرجينال وفى نفس الوقت لم تظهر اى أعراض لمرض الحمى القلاعية على هذه الحيوانات. كشفت الدراسة عن أن الإصابة بالانابلازما مارجينال معتدلة، فضلا عن انتشار مرض الحمى القلاعية بين الإبل في المملكة العربية السعودية، هذا وسيكون من الأهمية معرفة إذا كانت الجمال تلعب دوراً في نقل مرض الحمى القلاعية، وبالإضافة إلى ذلك يلزم إجراء دراسات لتقييم دور قمع المناعة لانابلازما مارجينالي للإصابة بمرض الحمى القلاعية في الجمال، على أية حال؛ من المستحسن تطبيق برنامج التطعيم لقطعان الجمال ضد مرض الحمى القلاعية لتجنب انتشار المرض بين الجمال وخاصة الحاملة للفيروس والذي يعيش معظمهم في اتصال بحيوانات المزرعة الأخرى القابلة للعدوى.

INTRODUCTION

In the Saudi Arabia, It is well known that camels are resistant to many infectious diseases, due to their physiological attributes, camels are the most suitable species of domestic mammals to be used under extremely arid conditions (Seri *et al.*, 2003; Bikaner, 2008). Despite the general reputation for hardiness and resilience, camels are, however, vulnerable to many infections (Wernery *et al.*, 2004) and parasitic agents (Bukachi *et al.*, 2003; Mohammed *et al.*, 2007).

Anaplasma is one of the most important parasites transmitted by at least 20 tick species (Marchette and Stiller, 1982), but mostly *Boophilus microplus* causing anaplasmosis (TFRC, 1996). Anaplasma parasite is responsible for a severe hemolytic disease, anaplasmosis; is usually caused by *Anaplasma marginale* (Kocan *et al.*, 2000). Anaplasmosis is an infectious, non contagious, tick born disease of domesticated and wild ruminants. Fever, progressive anemia, digestive disturbances, emaciation are the main characteristics of this disease (Kocan *et al.*, 2003). The disease has a worldwide distribution, particularly in tropical and sub-tropical regions. It has been also recorded in some temperate areas, the disease occur sporadically or as outbreaks leading to substantial significant economic losses (Smith, 1996).

Immunosuppressive effects are seem to be most of the time observed for various pathogenic agents, in particular in tick-borne diseases, as babesioses with other parasites in mice, or anaplasmosis (Persing, 1997). Studies of anaplasmosis in camels in KSA are sparse and little information had been provided.

Foot-and-mouth disease (FMD) is highly contagious viral disease that affects all species of cloven-hoofed animals and wildlife including cattle, buffalo, sheep, goat, pig, elephant, camel and deer, leading to severe economic loss of livestock industries worldwide (Somsak, 2004, Valarcher *et al.*, 2004). The Office International des Epizooties (OIE) code chapter on FMD includes camelids as being susceptible species to FMD, giving the impression that they are similar to cattle, sheep, goats and pigs in their potential involvement in the epidemiology of FMD (Wernery and Kaaden, 2004).

Amongst the camelidae, Bactrian camels and new world camelids have been shown to be susceptible to FMD virus (Larska *et al.*, 2008). FMDV is present in camels even without clinical signs and camel can be a source of infection for cattle and other animals. Two serotypes (O and A) of FMDV were detected in both species (camel and cattle) by RT-PCR in Egypt (El Hakim, 2005). The presence of antibodies to FMDV in camel sera was reported in Sharkia province Egypt, (Moussa *et al.*, 1970). Shiilegdamba *et al.* (2008) were reported 44 FMD outbreaks that affected cattle, sheep, goats, and camels in Mongolia.

During the 1981 outbreak of FMD at the Assam Zoo (India), a large number of wild ungulates including members of the family Camelidae, became infected (Sarma *et al.*, 1983). It has been reported that dromedaries can contract the disease following experimental infection and via close contact with FMD diseased livestock (Kitching, 2002). Virus isolation and ELISA are the gold standard tests for diagnosis of FMD (Alexandersen *et al.*, 2003). Antibodies to the polyproteins 3ABC are generally considered to be the most reliable indicators of FMDV infection (Mackay *et al.*, 1997).

Immunity of FMD in animals appears to be mainly dependent on serum neutralizing antibody levels present at the time of

exposure to infection. It is possible that breeding, age, nutrition and concurrent disease may play a role in influencing the magnitude of the antibody response to FMDV, in addition; certain concurrent disease present in the animal at the time of infection can reduce immune response (Muneer *et al.*, 1988).

During the course of parasitic infections, the host's immunologic response to FMD virus is often depressed (Sharpe *et al.*, 1982, Sharpe and Langley, 1983, Ahmad *et al.*, 1991, Scott *et al.*, 1977).

Although many of the clinical and pathologic features of the *Anaplasma marginale*, as well FMD are well recognized in animals, the role of immunosuppression of *Anaplasma marginale* in the outcome of FMD infection in camel is poorly studied.

The objective of this study was to investigate the incidence of *Anaplasma marginale* and FMD among camels in Saudi Arabia in viewing the effect of immunosuppression due to infection of camel with *Anaplasma marginale* on susceptibility of camel to FMDV infection.

MATERIALS and METHODS

Animals and area of study: The study were conducted on 138 Arabian (male and female) one-humped camels (Camelus dromedaries), 3~10 years old. The study was carried out in different middle regions of Al-Riyadh Province in the Kingdom of Saudi Arabia (Al-Riyadh,Thumamah, Dawadmi and Wadi Al-Dawasir).

Blood samples: Ten milliliter of blood were drained from each animal by Jugular Vein-puncture, then 5 ml of blood mixed with EDTA used for blood film and stained with Giemsa. The remaining (5ml) clotted blood was centrifuged at 3000 rpm. The serum was aspirated and stored at 20°C until used.

Microscopic examination (Thin blood film):

A small drop of fresh blood was put in the middle of one end of the slide, and spread right across the slide and then air dried. The slide was labeled using a pencil. Blood films were fixed in absolute methyl alcohol for 5 minutes, stained in 10% diluted Giemsa's stain for 45 minutes, and washed with distilled water and then dried.

The blood films were examined microscopically under oil immersion lens for the detection of blood parasites at 10×100 magnification. The parasite identification was done with the help of keys mentioned in the book titled "Helminths, Arthropods and Protozoa of Domestic Animals" (Soulsby, 1982).

ELISA Test: All serum samples were tested by **CHEKIT FMD-3ABC** (IDEXX Laboratories) is an indirect ELISA that detects antibodies to the FMDV 3-ABC polyprotein, according to the protocol recommended by the manufacturer instructions. Microtiter plates are supplied precoated with recombinant FMDV 3-ABC viral antigen. Dilution of the samples to be tested are incubated in the wells of these plates. Washing plates three times. A peroxidase-labeled anti-IgG conjugate was added that binds to the antibodies of the sample complexed with the 3ABC antigen. Washing plates three times, then the substrate was added to the wells. The optical density was measured at 450 nm. The diagnostic relevance of the result is obtained by comparing the optical density (OD) that develops in wells containing the samples with the OD from the wells containing the positive control.

RESULTS

Blood smears:-

Microscopically *Anaplasma marginale* appears as spherical granules near periphery of infected red blood cells in 32 (23.19%) examined blood samples (Fig.1). Parasiteamia was ranged between (3-5%).

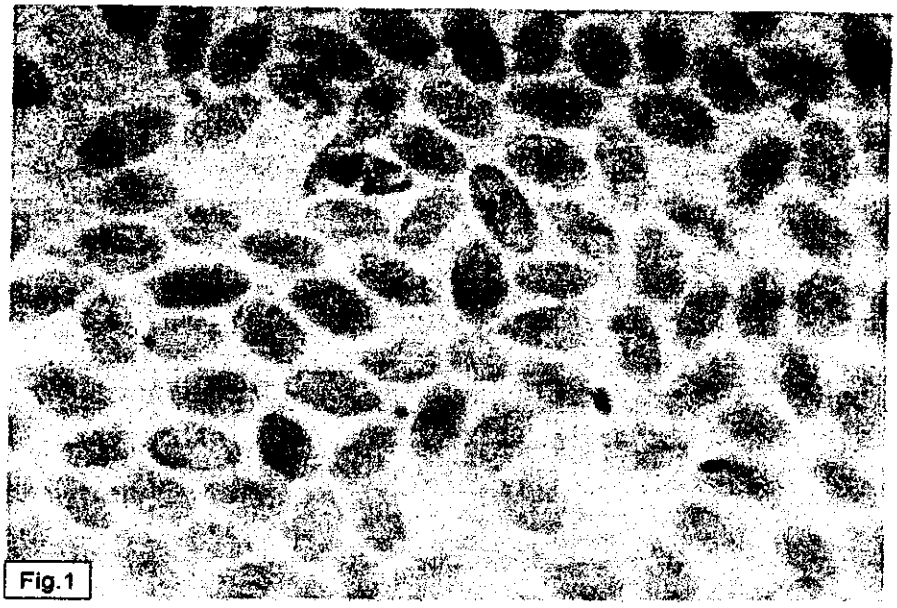

Fig. 1: Red blood cells infested with *Anaplasma marginal.* Giemsa stain. X=100

3ABC ELISA:

Nineteen out of 138 (13.76%) serum samples were positive to presence of 3-ABC-FMD antibodies. All FMD positive animals were positive to infection with *Anaplasma marginal.*

DISCUSSION

The clinical sings observed in infected camels with *Anaplasma marginale* were in agreement with those described by (Maghaddar, 2002; Sayed, 1998 and Higgins, 1984) as paleness of mucous membranes were exhibited the development of anemia. The presence of ticks on infected camels indicated that it is the important transmitters of anaplasma sp. (Mohammed *et al.*, 2007). Stained blood films in the current research revealed that *Anaplasma marginale* appears as spherical granules near periphery of infected erythrocytes, these results were similar to those shown by (Maghaddar, 2002). The positive percentage (23.19%) was in agreement with percentage in Nigeria (21.5%) where, hemoparasites (Babesia and Anaplasma) observed were *Anaplasma marginale* in camel (Rabana *et al.*, 2011).

The percentage of parasitemia ranged between 3-5% that agreed with Alsaad, (2009) who found 5-11% in camels. Increase body temperature may indicate liberation of endogenous pyrogens due to cellular lyses stimulating thermoregulatory centers of the hypothalamus (Higgin, 1986). The cause of anemia during blood parasitic infection may be multifactorial, due to the direct effect of the parasite to the infected erythrocytes or decrease the life span of RBCs and or suppression of hemopoitic system (Mahran, 2004).

FMD is also of great importance to Camelus dromedaries, because the disease is endemic in many countries, where *Camelus dromedaries* are reared. Saudi Arabia, with a dromedary population of nearly one million, annually imports approximately 6.5 million

livestock, mainly sheep and goats from Asia, Africa and Australia. Animals from Africa and Asia bring different FMDV serotypes which spread within the nomadic herds (Aidaros, 2002).

FMD remains the single most important animal disease, and *Camelus dromedaries* inhabit countries in North and East Africa, the Middle and Far East as well as in South America where FMD is endemic and the detection of antibody to non structure protein (NSP's) must indicate infection rather than vaccination (Wernery and Kaaden, 2004).

It has been reported that dromedaries can contract the disease following experimental infection and via close contact with FMD diseased livestock (Kitching, 2002). Nineteen out of the 138 (13.76%) samples were positive to presence of FMD antibodies, this result agreement with the presence of antibodies to FMDV in 39 camel sera tested in Sharkia province; Egypt (Moussa *et al.*, 1970) and between April 2000 and July 2002, camel infected in Mongolia (Shiillegdamba *et al.*, 2008), in addition; FMDV is present in camels even without clinical signs and camel can be a source of infection for cattle and other animals, (El Hakim, 2005). But disagree with (Wernery and Kaaden, 2004); the prevalence varied widely and that may be depending on the serologic test used in the survey (Moussa *et al.*, 1970).

It seems that the examined camels in our study which infected by *Anaplasma marginale* may become susceptible to infection by FMD and infected through close contact with FMD diseased livestock. Outbreaks of FMD repeatedly occur among cattle, sheep and goats in various regions of SaudiArabia (Hafez *et al.*, 1993).

Camels are frequently moved across the desert inside Saudi Arabia in an area that experienced FMD outbreaks in cattle and small ruminants so camels may play a possible role in the transmission of FMDV and may carry FMDV over very long distances and across borders. Anaplasma infection is the ensuing immunosuppression that may lead to secondary infections (Grova *et al.*, 2011; Dumler and Broqui, 2004).

In conclusion; our study revealed infection of *Anaplasma marginale* may be leads to increase susceptibility of camels to infection with FMDV. It would be of great interest to know if dromedaries play a role in transmitting the FMDVs, in addition; further studies are required to evaluate the possible immunosuppression role of Anaplasma marginale to FMDVs infections in camels. Application of vaccination program for camel populations against FMD in KSA is recommended to avoid the spread of FMDV by carrier camels which live mostly in contact with other susceptible animals.

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