STUDIES ON CAUSES OF ABORTION IN MAGHRABIAN CAMELS

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Abstract

History of abortion, stillbirths and death of camel calves within 6 months of birth were appeared in the year of 2013 in Maghribian camels in a farm in Mersa Matrouh governorate. Whole blood and serum samples were collected from 34 camels ≥ 5 years and from 19 camels < 5 years. Blood films prepared for detection of blood parasites by Giemsa stain. Serum samples used for detection of *Toxoplasma* antibodies by slide Toxo-Latex agglutination test (LAT) and *Chlamydophilla* spp. antibodies were detected by complement fixation test (CFT). Fifteen internal organs (liver, heart, lung and spleen) from aborted calves and twenty vaginal swabs from she camels that aborted were collected for isolation of chlamydiae via inoculation in embryonated chicken eggs (ECE). *Toxoplasma* antibodies, *Theileria*, *Anaplasma* and mixed infection (*Theileria* and *Anaplasma*) were detected in 70.6%, 26.5%, 17.6% and 8.8% of camels ≥ 5 years, while they were detected in 42.1%, 26.3%, 10.5% and 5.3% of camels < 5 years respectively. Examined serum samples by CFT showed 27% were positive for *C. psittaci* antibodies. Chlamydial inclusion bodies were detected in 45% and 20% of vaginal swabs and internal organs respectively by chicken embryo inoculation. Camels were treated with coliprim, butalex, imizol and alamycin which repeated routinely every 6 months. Number of aborted fetuses and stillbirths were decreased in years 2014 and 2015, while the number of still alive camel calves were increased in the same 2 years. No camel calves were dead within 6 months of birth in year 2015.

Key words: Camels, Abortion, *Toxoplasma*, *Theileria*, *Anaplasma*, *Chlamydia*.

INTRODUCTION

Camels (*Camelus dromedarius*) are important multipurpose animals in many countries all over the world including Egypt. They are a source of meat, milk and hides production. Camels affected with various protozoal parasites such as *Toxoplasma*, *Theileria* and *Anaplasma* showed many symptoms including abortion, fetal death, infertility and decrease in milk yield.

Toxoplasa gondii is a heteroxenous coccidian parasite. The definitive hosts are domestic cats and various species of wild felids. The intermediate hosts are mammals and birds (Elamin *et al*., 1992). *T. gondii* cause abortion, neonatal death and faetal abnormalities in animals and human (Hayde and Pollak, 2000 and Trees and Williams, 2005). Toxoplasmosis is a globally distributed zoonosis with serious impact on unborn fetuses and also immunosuppressed individuals (Klun *et al*., 2006). In Egypt Michael *et al*. (1977) and Hilali *et al*. (1998) reported toxoplasmosis in camels.

Theileriosis and anaplasmosis are tick born blood protozoal disease affecting camels. *Theileria* infection reported in camels in Egypt by El-Refaii *et al*. (1998) and El-Fayoumy *et al*. (2005). Ismael *et al*. (2014) found *Theileria* infection in Saudi Arabia in camels suffered from symptoms included abortion and/or infertility. *Anaplasma* infection recorded in camels in Saudi Arabia and Tunisia (Bastos *et al*., 2015 and Belkahia *et al*., 2015). *A. marginale* is highly pathogenic and abortion is one of the clinical symptoms appeared in ruminants and associated with fetal infection (Urdaz-Rodríguez *et al*. 2009 and Hairgrove *et al*. 2015).

Chlamydophila spp. is a genus of Gram negative bacteria which has been reported as pathogenic in camels. *Chalmydophila* spp. is an ubiquitous parasite with mixed infections occurring frequently (Reinhold *et al*., 2011). Chlamydophilosis is a major cause of abortion in domestic ruminants and caused by *Chlamydophila* spp. (Aljumaah and Mansour, 2012). Camels could be infected with *Chlamydophila*, but most infected camels appear healthy so, they can play a very important role in transmission of this infection to contact animals.

The aim of the present work is to study the causes of the case history of abortion appeared in Maghribian camels in a farm in Mersa Matrouh governorate and how to control it.

MATERIALS AND METHODS

History in the farm:

In the year of 2013 a farm of Maghribian camels in Mersa Matrooh governorate suffered from abortion (5/19), stillbirths (3/19) and death of newly born camel calves within 6 months of birth (10/19). The number of still alive camel calves were only 1 from 19.

Sample collection:

At the beginning of the year 2014, blood samples for serum separation were collected in clean vacuotainer tubes from jugular vein of 34 camels ≥ 5 years and 19 ones < 5 years. Another blood samples from the same camels were collected in another clean tubes containing sodium salt of EDTA anticoagulant. The collected samples were sent to laboratory in ice box containing ice packs. For detection of chlamydial inclusion bodies, 15 internal organs (liver, heart, lung and spleen) from

aborted calves were collected. The organs were kept in clean labeled plastic bags in deep freezer until used for egg inoculation. Also, 20 vaginal swabs were collected from aborted camels through sterile endocervical swabs. These swabs were kept into sterile tubes containing PBS and used for egg inoculation.

Methodology:

Parasitological examination:

Sera were separated by centrifugation at 3000 rpm for 10 minutes and stored at -20ºC until use. Rapid qualitative detection of *Toxoplasma* antibodies was applied by slide Toxo-Latex agglutination test (LAT) using Toxo Latex kit (Cam Tech Medical, 4 Stable Crest, Bradford, West Yorshire, BD2 IE7, United Kingdom). The kit was used strictly following manufacturer instructions. Blood films were prepared, stained with Giemsa stain and examined microscopically for detection of blood parasites.

Bacteriological examination:

1- Detection of *C. psittaci* antibodies in the collected serum samples by complement fixation test (CFT):

CFT was conducted according to Edwin and Nathalie (1979) using amboceptor (anti-sheep red blood cell); reference antiserum and antigen of chlamydiae for CFT (*C. psittaci* CF test Reagent "Seiken") supplied commercially from Denka Sieken Co., Ltd., Tokyo, Japan. Controls were included throughout the entire testing (complement control, positive known serum control and antigen control).

2- Preparation of samples for inoculation of chicken embryo:

Sterile vaginal swabs were collected from cases of abortion according to Black (1997). These swabs were placed into sterile tubes containing PBS (pH 7.5). The internal organs of each aborted camels were pooled and grinded in sterile mortar with sterile sand under aseptic conditions with the addition of PBS (pH 7.5) and then centrifuged for 15 min. at 3000 rpm. A clear supernatant fluid was transferred under aseptic condition using a sterile pipette to another centrifuge tube (Andersen, 1998). A stock solution of antibiotics (Sigma–Aldrich, MO, USA); streptomycin (1 mg/ml), vancomycin (1 mg/ml) and nystatin (100 units/ml) were added to inhibit micro-organisms other than chlamydiae. The final supernatant was used for inoculation of the embryonated chicken egg through yolk sac route.

3- Chicken embryo inoculation and staining of yolk sac using Gimenez stain:

Procedure for isolation according to Pierre and Michel (1993). Seven days old specific pathogen free (SPF) fertile chicken eggs were used for detection of chlamydiae. Thirty five samples (15 internal organs and 20 vaginal swabs) were inoculated into the egg yolk sac and the inoculated eggs were incubated at 37°C in a humidified incubator. Non inoculated control eggs were labeled and incubated beside the inoculated eggs. The eggs were candled on a daily basis and the eggs that died

within 3 days post inoculation were discarded while those died after day 3 to day 10 are opened. The yolk sac membranes were harvested and stained by Gimenez stain (Gimenez, 1964). Embryos of specific deaths were examined for gross changes and lesions specific for chlamydial infection.

Treatment:

A program of treatment of camels started at the beginning of 2014 and repeated routinely every 6 months. Camels treated for *Toxoplasma* infection with coliprim by intramuscular injection with 2.5 ml/40 kg body weight for 3 successive days (each 1 ml contains 200 mg sulphadiazine sodium and 40 mg trimethoprim). For *Theileria* infection, camels treated with butalex by intramuscular injection with 1 ml/20 kg body weight, 2 doses 72 hours apart (each 1 ml contains 50 mg buparvaquone) and for *Anaplasma* infection, they injected with imizol subcutaneously with 2 ml/100 kg body weight (each 1 ml contains 85 mg imidocarb dipropionate). For *Chlamydia* infection, camels treated with alamycin by intramuscular injection with 1 ml/10 kg body weight (each 1 ml contains 200 mg oxytetracycline).

RESULTS

Table (1) showed that *Toxoplasma* antibodies were investigated in 70.6% (24/34) and 42.1% (8/19) of camels ≥ and < 5 years respectively.

Concerning blood parasites in camels ≥ 5 years, infection rates with *Theileria* (erythrocytic and/or schizonts), *Anaplasma marginale* and mixed infection were 26.5% (9/34), 17.6% (6/34) and 8.8% (3/34) respectively. Camels < 5 years showed infection with the same blood parasites with rates of 26.3% (5/19), 10.5% (2/19) and 5.3% (1/19) respectively. After treatment, blood parasites were not detected in old and young camels.

Table 1. Detection of *Toxoplasma gondii* antibodies and blood parasites in camels ≥ and < 5 years.

	Toxoplasma gondii by latex agglutination test	Blood parasites detected					
		Theileria		*Anaplasma marginale*		Mixed infection*	
		% and No. before treatment	% and No. after treatment	% and No. before treatment	% and No. after treatment	% and No. before treatment	% and No. after treatment
Camels ≥ 5 years	70.6% (24/34)	26.5% (9/34)	0% (0/34)	17.6% (6/34)	0% (0/34)	8.8% (3/34)	0% (0/34)
Camels < 5 years	42.1% (8/19)	26.3% (5/19)	0% (0/19)	10.5% (2/19)	0% (0/19)	5.3% (1/19)	0% (0/19)

* *Theileria* and *Anaplasma marginale*

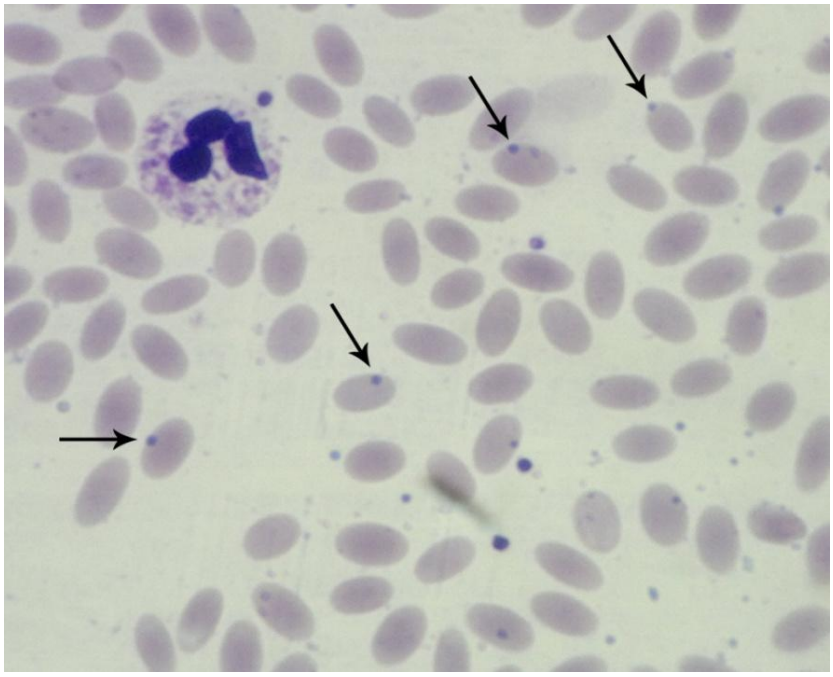

Fig.1. *Anaplasma marginale* inside RBCs in Giemsa stained blood film of camel. (X 1000)

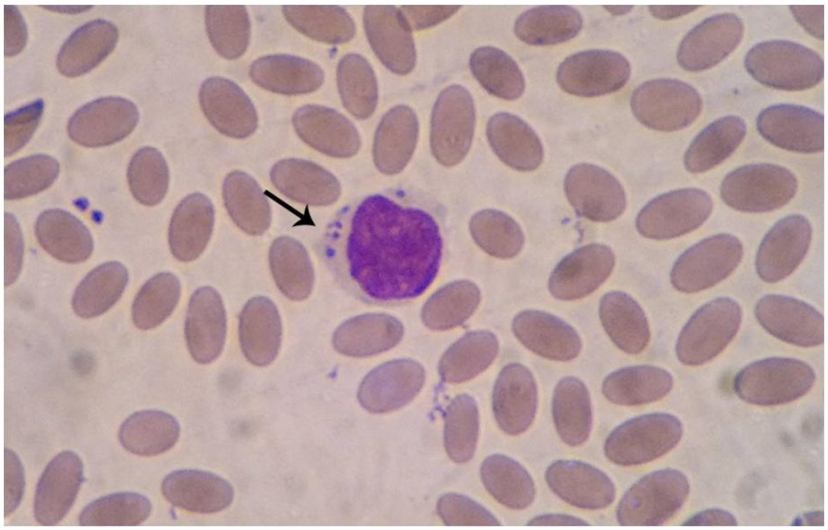

Fig. 2. Schizonts of *Theileria* (Kbb) inside lymphocyt in Giemsa stained blood film of camel. (X 1000)

Detection of *C. psittaci* antibodies in the collected serum samples by complement fixation test (CFT):

Table (2) showed that 28.3% (15/53) of examined serum samples were positive for the presence of *C. psittaci* antibodies using CFT. Positive samples titer with 1/32 were detected in 8.8% (3/34) and 15.8% (3/19) in camels ≥ 5 and < 5 years respectively.

Positive samples titer ≥ 1/64 were detected in 8.8% (3/34) and 31.6% (6/19) in the same camel ages respectively.

Table 2. Detection of *Chlamydia psittaci* antibodies in the collected serum samples by complement fixation test (CFT).

Total No. of examined samples (53)	Positive samples titers	Total positive No.	Positive %
34 samples ≥ 5 years	1/32	3	8.8%
19 samples < 5 years	1/32	3	15.8%
34 samples ≥ 5 years	≥ 1/64	3	8.8%
19 samples < 5 years	≥ 1/64	6	31.6%
Total		15	28.3%

Detection of chlamydiae antigen using egg inoculation:

Using Gimenez staining, chlamydial inclusions appeared in the collected yolk sac membranes as small, rounded red dots (Fig.3). The infected egg embryos appeared dwarfed with presence of hemorrhagic spots in the head and toes (Fig. 4).

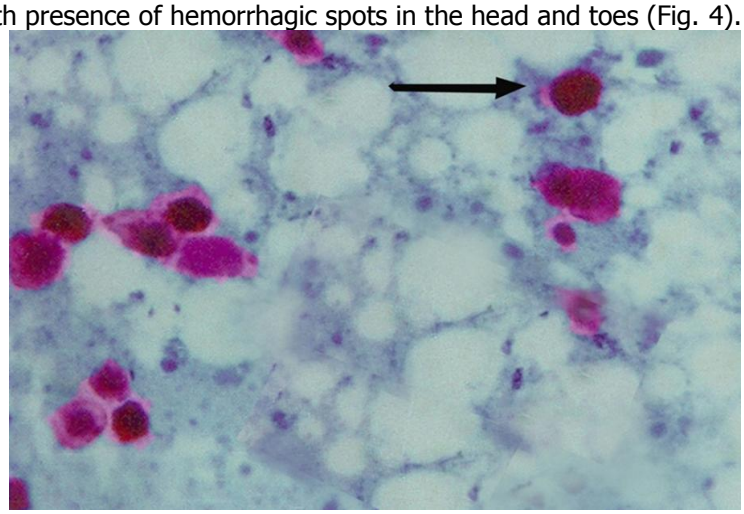

Fig. 3. The inclusion bodies in the infected yolk sac membrane stained with Gimenez stain. X1200.

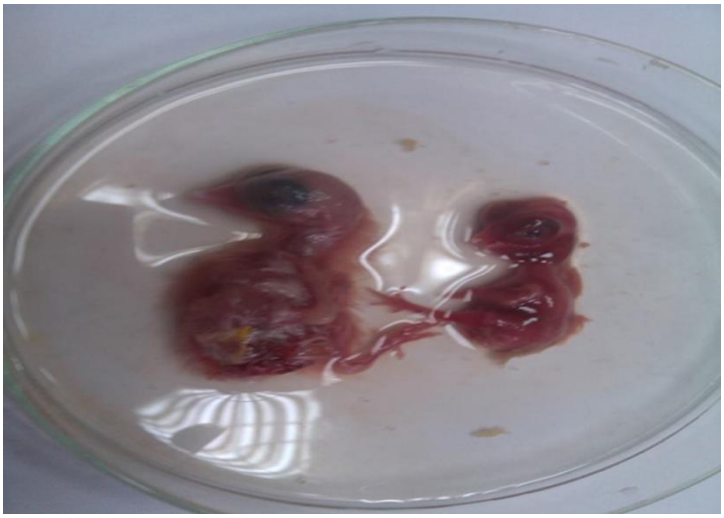

Fig. 4. The growth abnormalities, dwarfism and congestion of chicken embryo.

Table (3) showed that the chlamydial inclusion bodies within the examined samples in yolk sac by Gimenez stain detected in 45% (9/20) of vaginal swabs and in 20% (3/15) of internal organs.

Table 3. Direct detection of chlamydial inclusion bodies within the examined samples in yolk sac using Gimenez stain.

Total No. of examined samples (35)	No. of negative samples	No. of positive samples	Positive %
Vaginal swabs (20)	11	9	45%
Internal organs (15)	12	3	20%
Total	23	12	34.3%

Table (4) illustrated history of abortion, stillbirths, death of camel calves within 6 months of birth and the still alive camel calves in 2013 and after treatment in 2014 and 2015. Number of aborted fetuses was 5 (26.3%) in year 2013 and decreased during 2014 and 2015 to become 4 (20%) and 1 (5.3%) respectively. Number of stillbirths decreased from 3 (15.8%) in 2013 to become 1 (5% and 5.3%) in the next 2 years. Number of camel calves dead within 6 months of birth in years 2013 and 2014 were 10 (52.6%) and 11 (55%) respectively while no camels dead in 2015. Number of still alive camel calves in the farm improved as it increased from 1 (5.3%) in 2013 to become 4 (20%) and 17 (89.5%) during the next 2 years.

Table 4. History of abortion, stillbirths, death of camel calves and still alive ones in the farm during years from 2013 till 2015.

Year	No. of Pregnant camels	No. of aborted fetuses	%	No. of Stillbirths	%	No. of camel calves dead within 6 months of birth	%	No. of still alive camel calves	%
2013	19	5	26.3%	3	15.8%	10	52.6%	1	5.3%
2014*	20	4	20%	1	5%	11	55%	4	20%
2015	19	1	5.3%	1	5.3%	--	0%	17	89.5%

*The program of treatment started at the beginning of 2014 and repeated every 6 months

DISCUSSION

History of abortion, stillbirths and death of camel calves within 6 months of birth appeared in a farm in Mersa Matrouh in year of 2013. Laboratory diagnosis indicated the presence of *Toxoplasma* antibodies and infection with *Theileria*, *Anaplasma* and *Chlamydia*. Many authors recorded those infections in camels in different countries which cause the same history. Hayde and Pollak (2000), Trees and Williams (2005) and Hide *et al*. (2009) recorded that *T. gondii* cause abortion, vertical transmission, neonatal death, faetal abnormalities and congenital disease in animals and human. Ismael *et al*. (2014) found that *Theileria* infection in camels cause abortion and/or infertility. Baek *et al*. (2003) showed that abortion, stillbirths and vertical transmission could be occur in cows infected with *Theileria* spp. Urdaz-Rodríguez *et al*. (2009) and Hairgrove *et al*. (2015) stated that *Anaplasma* is highly pathogenic for ruminants and cause abortion and fetal death.

In the studied farm, there was no history of previous treatment for toxoplasmosis, so the detection of *Toxoplasma* antibodies by LAT indicated the presence of previous *Toxoplasma* infection or recent infection. *Toxoplasma* antibodies were higher in camels ≥ 5 years (70.6%) than those < 5 years (42.1%). Elamin *et al*. (1992) and Hamidinejat *et al*. (2013) recorded the occurrence of *T. gondii* antibodies was greater in older camels and that might be due to the longer period of exposure to infection in older camels compared to young ones. Also, it may be due to the vertical transmission from mother to fetus through placenta (Ebbesen, 2000).

In the present study, there is no great difference between rate of infection with *Theileria*, *Anaplasma* and mixed infection with them in adult and young camels before treatment. After treatment the infection rate decreased to 0% in both adult and young camels.

The present study was primarily undertaken to determine the prevalence of chlamydiosis in camels in Egypt. The results suggest that camels are an ideal reservoir of *Chlamydia* species and thus shed the organisms in their excreta. The shedding of *Chlamydia* by wild birds throughout the Egyptian habitat may trigger another zoonotic potential to humans existing at their vicinity. The organism is also important from a zoonotic standpoint, particularly as an occupational hazard.

Ali *et al*. (2012) reported that *C. abortus* may be responsible for the spreading of the ovarian hydrobursitis syndrome in dromedaries. In the United Arab Emirates, Wernery and Wernery (1990) detected anti-chlamydial antibodies in the sera of both breeding and racing camels, with respective prevalence rates of 24 and 15%. In Saudi Arabia, a serological prevalence of 19.4% was reported for chalmydiosis in camels with a higher prevalence in females than males (Hussein *et al*., 2008). In Libya, Elzlitne and Elhafi (2016) showed that 12.25% of tested camels were positive for anti-*Chlamydia* antibodies and it was higher in females (14.0%) than males (5.0%). Chlamydial infection has been associated with abortion in New World camelids, and the prevalence of chlamydiosis was higher in females than in males (Wernery and Wernery, 1990 and Ali *et al*., 2012).

Culture of chlamydiae is difficult and infrequent because of the obligate intracellular nature of the bacteria and the hazard exposed to researchers (Messmer *et al*., 2009). Cell culture or egg inoculation is the gold standard for diagnosis of *Chlamydia* spp. Isolation of viable *Chlamydia* spp. requires infection of embryonic egg or cell culture (Condon and Oakey, 2007).

After treatment program followed in the present work, the number of aborted fetuses decreased from 26.3% in year 2013 to become 20% and 5.3% in 2014 and 2015 respectively. The stillbirths also decreased from 15.8% to 5% and 5.3% in the same years. Percent of camel calves dead within 6 months of birth were 52.6% and 55% in 2013 and 2014 respectively and decreased to 0% in 2015. On the other hand, the percent of still alive camel calves increased from 5.3% in 2013 to 20% and 89.5% in 2014 and 2015 respectively.

In the present study, the program of treatment included trimethoprim plus sulphadiazine, buparvaquone, imidocarb dipropionate and oxytetracycline succeeded in treatment and control of toxoplasmosis, theileriosis, anaplasmosis and chlamydiosis. Bawazeer and Nizamuddin (2008) and Felix *et al*. (2014) tried trimethoprim in combination with sulfonamide in treatment of toxoplasmosis with various therapeutic efficacies. Singh *et al*. (1993) and Muraguri *et al*. (2006) reported the use of buparvaquone with success in treatment of theileriosis in cattle. McHardy and Simpson (1974) and Patarroyo *et al*. (1982) used imidocarb dipropionate in the treatment of anaplasmosis.

It could be suggested that the programme of treatment with trimethoprim plus sulphadiazine, buparvaquone, imidocarb dipropinate and oxytetracycline may offer an effective procedure to control history of abortion, stillbirths and death of camel calves caused by toxoplasmosis, theileriosis, anaplasmosis and chlamydiosis. Control of those diseases can help camels to restore their activity and consequently regain their health and increase the economics of animal production in the farm.

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دراسات عن مسببات الإجهاض في الإبل المغربي

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حدثت حالات إجهاض وخروج أجنة نافقة ونفوق لإبل مولودة خلال الستة أشهر الأولي من الولادة في مزرعة للإبل المغربي بمحافظة مرسى مطروح خلال عام ٢٠١٣م. تم جمع عينات دم كامل وسيرم من ٣٤ إبل أعمار أكبر من أو يساوي ٥ أعوام ومن ١٩ إبل أقل من ٥ أعوام وذلك للفحص لطفيليات الدم بصبغة الجيمسا والكشف عن الأجسام المناعية للتوكسويلازما والكلاميديا. كما تم جمع عينات من أعضاء داخلية (كبد – قلب – رئة – طحال) من عدد ١٥ أجنة وكذلك ٢٠ مسحة مهبلية من إناث الإبل المجهضة وذلك لعزل الكلاميديا عن طريق الحقن في البيض المخصب. بالفحص الطفيلي وجدت أجسام مناعية للتوكسوبلازما (٧٠،٦%) وطفيل الثيليريا (٢٦،٥%) والأنابلازما (١٧،٦%) وإصابة مختلطة من الثيليريا والأنابلازما معاً (٨،٨%) في الإبل أكبر من أو يساوي ٥ أعوام. وجدت نفس الإصابات بنسب ٤٢،١% ، ٢٦،٣% ، ١٠،٥% ، ٥،٣% في الإبل أقل من ٥ أعوام على التوالي. بفحص عينات السيرم باستخدام إختبار المثبت المكمل وجد أن ٢٧% من العينات إيجابية للأجسام المناعية للكلاميديا سيتاسي. وجدت أجسام محورية للكلاميديا في ٤٥% من المسحات المهبلية و في ٢٠% من الأعضاء الداخلية باستخدام الحقن في أجنة الدجاج. تم علاج الإبل بالمزرعة دورياً كل ٦ أشهر بالكوليبريم ، البيوتالكس ، الإيميزول ، الألاميسين. وجد إنخفاض في عدد الإجهاضات والأجنة النافقة داخل الرحم في أعوام ٢٠١٤ ، ٢٠١٥م بينما إزداد عدد الإبل الصغيرة التي استمرت على قيد الحياة في نفس الأعوام. لم ينفق إي عدد من الإبل الصغيرة خلال الستة أشهر الأولى من ولادتها خلال عام ٢٠١٥م.