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Serological Prevalence of Abortifacient Agents in Female *Mijaheem* Camels (*Camelus dromedarius*) in Saudi Arabia

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ABSTRACT

Sera from 378 female *Mijaheem* camels of different age groups were collected from different parts of the country. All of them were simultaneously tested serologically for specific antibodies against brucellosis, chlamydiosis and coxiellosis while 368 of the same serum samples were also tested for toxoplasmosis and neosporosis, using indirect enzyme-linked immunosorbent assays. The results were statistically analyzed to determine the prevalence rate of each of these abortifacient agents and the association of the geographical location and age of the animal with the prevalence. The overall serological prevalence was 16.67% for brucellosis, 49.18% for toxoplasmosis, 13.32% for neosporosis, 10.05% for chlamydiosis and 42.36% for coxiellosis. Statistical analysis revealed significant associations between the serological prevalence of each of the studied abortifacient agents with the location of the animal, and there was also a significant association with age in the case of toxoplasmosis and chlamydiosis. The results indicate that the causative agents of brucellosis, toxoplasmosis, neosporosis, chlamydiosis and coxiellosis are widespread among indigenous *Mijaheem* camels in Saudi Arabia, and that their prevalence varied with region and age group. These different agents should therefore be considered in the differential diagnosis of infectious abortion in Saudi Arabian camels.

Keywords: Camels, reproduction, abortion, disease, prevalence.

Reproductive failure in farm animals may be caused by infectious or non-infectious (toxic or genetic) agents, the former being much more common. In addition to early embryonic death and abortion, abortifacient infections may also cause stillbirth, fetal mummification, birth of weak or deformed neonates and reproductive disorders in dams. Many agents that commonly cause abortion in farm animals are also zoonotic.

Pregnancy loss involving both infectious and non-infectious etiologies is common in camelids, with infectious abortion rates ranging between 10% to more than 70% in some areas (Tibary *et al*., 2006; Tibary, 2012). The commonest causes of infectious abortion in dromedary camels are brucella spp. (*Br. meletensis* and *Br. abortus*) and *Trypanosoma evansi* (Gutierrez *et al*., 2005; Tibary *et al*., 2006; Gwida *et al*., 2012). Other causes include leptospirosis (Wernery and Wernery, 1990), *Toxoplasma gondii* (Hussein *et al*., 1988; Elamin *et al*., 1992; Hilali *et al*., 1998; Serrano-Martinez *et al*., 2007), *Chlamydia abortus* (Hussein *et al*., 2008; Elzlitni and Elhafi, 2016; Osman *et al*., 2016; Zaher *et al*., 2017), *Neospora caninum* (Hilali *et al*., 1998; Serrano- Martinez *et al*., 2007; Hamidinejat *et al*., 2013), *Listeria monocytogenes* (Tibary, 2012), Campylobacteriosis (Wernery and Ali, 1989; Tibary *et al*., 2006); BVD virus (Tibary, 2012), equine rhinitis A virus (Wernery *et al*., 2008), *Anaplasma marginale* (Osman *et al*., 2016), theileriosis (Ismael *et al*., 2014), sarcosporidiosis (Tibary, 2012) and *Coxiella burnetii* (Jarelnabi *et al*., 2018). Of these abortion-causing organisms, five species, namely *T. gondii*, *N. caninum*, *Ch. abortus* and *C. burnetii* as well as brucellosis (*B. abortus* or *B. meletensis*) have been previously reported or suspected to cause abortion in one or more species of farm animals in Saudi Arabia. However, their prevalence among the Kingdom's livestock is largely unknown. The present study was undertaken to determine the serological prevalence of these organisms in female *Mijaheem* camels, the commonest camel ecotype indigenous to Saudi Arabia.

MATERIALS AND METHODS

Animals

Three hundred and seventy-eight dromedary camels were randomly selected from naturally grazing herds, animal enclosures and farms in the Central, Northern, Western, Eastern and Southern regions of Saudi Arabia. All of them were females *Mijaheem* camels. They belonged to three age groups: group I: <2 yr, group II: 2 - < 4 yr and group III > 4 yr.

All of the sampled camels were apparently healthy and none of the adults was pregnant at the time of sampling. Also, none of the camels was vaccinated against any of the tested abortifacient agents.

Serum samples of camels from the Central region were collected from Al-Kharj, ad-Dawadmi, Huraimila'a and Darma. Those from the Northern region were collected from Ha'il, Al-Jaouf and surrounding villages. Samples from the Western region were collected from herds along Um al-jumum road south of Makkah, and from Beish and Al-Laith, while those from the Southern region were collected from villages around Jazan and Asir provinces. Samples from the Eastern region were collected from Hijrat Tamani and Alhasa region.

Serological Tests

7-10 mL blood samples were collected from each camel by jugular venipuncture into plain vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ, USA). The samples were allowed to clot for 4 h at room temperature, and the sera were separated by centrifugation at 1,500×*g* for 15 min and stored at −20°C. Contaminated or hemolyzed samples were discarded and replaced. Indirect enzyme-linked immunosorbent assay (*i*ELISA) kits (IDEXX Switzerland AG, Stationsstrasse 12, 3097 Liebefeld-Bern, Switzerland) were used to screen the serum samples for specific IgG antibodies to inactivated antigens of brucella spp. (*B. abortus/B. meletensis*), *T. gondii*, *N. caninum*, *Ch. abortus*, and phases I and II purified *C. burnetii*, antigens. A horse-radish peroxidase (HRP)-conjugated goat anti-camel IgG (Triple J. Farms, 777 Jorgensen Place, Bellingham, WA 98226, USA) was used to detect positive sera. The tests were performed in microtiter plates pre-coated with the respective antigen and the manufacturer's instructions were strictly followed. Reference positive and negative sera were included in each test plate. The optical density (OD) of each sample was determined spectrophotometrically at 450 nm using microtiter plate reader and compared with the optical densities of the positive and negative reference sera. The optical density percentage (OD%) which is an indirect measure of antibody concentration was determined using the following equation:

$$\text{Percent O.D. of the sample} = \frac{100(S-N)}{(P-N)}$$

where, S, P and N are the O.D. value of the test, positive control and negative control sera, respectively. Samples giving OD% value equal or exceeding that specified by the manufacturer (80% for brucellosis and 40% for other infectious agents) were considered positive. Those with OD% values less than that given by the manufacturer were considered negative.

All 378 serum samples were simultaneously tested for antibodies against brucellosis, *Ch. Abortus* and *C. burnetii*. 368 of the same samples were tested for antibodies against *T. gondii* and *N. caninum*.

Statistical Analysis

The data were analyzed with the incidence of antibodies against each of the studied abortifacient agents coded as a binary dependent variable (0 for sero-negative and 1 for seropositive animals). Frequencies and means of prevalence and the ELISA titration results were computed for location and age, using Statistical Analysis System Version 9.1 software for windows. The differences in these variables between positive and negative samples were analyzed using Chi-square tests. Logistic regression models were used to examine the associations of location and age with the incidence of each abortifacient infection, and the associations were considered to be significant when $p<0.05$.

RESULTS AND DISCUSSION

The present study is the first attempt to determine, on a countrywide scale, the serological prevalence of five infectious agents that may cause abortion and other forms of reproductive failure in camels in Saudi Arabia, namely

Table 1: Optical Density Seroprevalence of Abortifacient Agents in Saudi Arabian Najdi Camels

Aborti-Facient Agent	Age Group	Region									
		Central		Eastern		Northern		Western		Southern	
		-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve
Brucella spp.	I	18	4	24	1	27	3	17	8	22	1
(*B. abortus/*	II	26	5	19	6	18	0	16	9	22	1
B. meletensis)	III	35	6	18	7	10	7	21	4	22	1
	Total	79	15	61	14	55	10	54	21	66	3
	(%)		15.96%		18.67%		15.38%		28%		4.35%
Toxoplasma	I	8	14	19	6	17	13	8	17	7	16
Gondii	II	16	15	19	6	8	10	3	22	1	22
	III	26	5	17	8	12	5	23	2	3	20
	Total	50	34	55	20	37	28	34	41	11	58
	(%)		40.48%		26.67%		43.08%		54.67%		84.06%
Neospora caninum	I	18	4	25	0	30	0	16	9	21	2
	II	31	0	25	0	14	4	9	16	22	1
	III	31	0	25	0	17	0	21	4	14	9
	Total	80	4	75	0	61	4	46	29	57	12
	(%)		4.76%		0%		6.15%		38.67%		17.39%
Chlamydia abortus	I	21	1	25	0	26	4	25	0	23	0
	II	24	7	18	7	16	2	25	0	23	0
	III	35	6	20	5	16	1	23	2	20	3
	Total	80	14	63	12	58	7	73	2	66	3
	(%)		14.89%		16%1		10.77%		2.67%		4.35
Coxiella burnetii	I	20	2	21	4	5	25	14	11	16	7
	II	3	28	14	11	0	18	25	0	20	3
	III	18	23	21	4	11	6	15	10	17	6
	Total	41	53	56	19	16	49	54	21	53	16
	(%)		56.38%		25.33%		75.38%		28%		23.19%

Brucella spp, *T. gondii*, *N. caninum*, *Ch. abortus* and *C. burnetii*.

The numbers of animals tested, their sampling locations and age groups are summarized in Table 1. Mean, minimum and maximum O.D. percentage values among the seropositive animals are given in Table 2. Among all of the serologically positive camels, only 11 were positive for one abortifacient agent and remainder were positive to 2 - 4 of these agents.

Table 2: Percent Optical Density in Camels' Sera Positive for Abortifacient agents

	Overall Percent Optical Density (O.D. %)			
	Prevalence (%)	**Minimum**	**Maximum**	**Mean ±SE**
Brucella spp	16.67%	80.00	417.00	210.00±12.55
T. gondii	49.18%	40.00	372.00	111.86±6.14
N. caninum	13.32%	40.00	93.00	42.11±2.86
Cl. abortus	10.05%	41.00	143.00	60.47±3.69
C. burnetii	41.80%	40.00	293.00	94.47±4.42

As can be seen from these tables, 63 out of 378 camels tested for brucellosis, were serologically positive, giving an overall prevalence of 16.67%, and the highest regional prevalence (29.33%) was recorded in the western region and least (4.35%) in the southern region. The O.D. percentage among positive camels ranged between 80-417%, with a mean value of 210+12.55%. There was a highly significant association of the location with seroprevalence of brucellosis (x^2 = 14.80, p<0.0051) while a non-significant (x^2 = 12.86, p <0.0119) association of age with seroprevalence was recorded. The overall serological prevalence of camel brucellosis in the present study (16.67%) was higher than that previously reported in Saudi Arabia (Hashim *et al*., 1987; Radwan *et al*., 1995; Hegazy *et al*., 2004; Alshaikh *et al*., 2007a) and elsewhere (reviewed by Radwan *et al*., 1992; Mekonnen, 2016) but comparable with that reported in southern Jordan (Dawood, 2008). No attempt was made during the present study to determine the species of brucella infecting the camels.

However, these animals are known to be susceptible to both *B. abortus* and *B. meletensis* (Cooper, 1991; Gwida *et al*., 2012) and both of these organisms was previously isolated from camels (Radwan *et al*., 1992; Alshaikh *et al*., 2007b; Mekonnen, 2016). On the other hand, no studies are available on the epizootiology, economic consequences and public health impact of camel brucellosis. Abbas and Agab (2002) stated that the seroprevalence of camel brucellosis fell into two distinct categories: a low prevalence of 2-5% in nomadic or naturally grazing camels and a high prevalence of 8-15% in intensively or semi-intensively reared camels. Radwan *et al*. (1992) reported prevalence rates of 4.3% in camels raised in the backyards of private homes and 8.6% in those raised in large camel farms, with an overall seroprevalence of 8%. These authors isolated *B. meletensis* biovars 1 and 2 from milk samples of camels in Riyadh region. Using PCR, Alshaikh *et al*. (2007a) detected *B. abortus* in the sera of Saudi camels.

Out of 368 camels tested for toxoplasmosis, 181 (49.18%) camels were positive for anti-*T. gondii* antibodies. An exceptionally high seropositivity (84.06%) was recorded in camels in the southern region, followed, in descending order, by the western, central, northern and eastern regions. The O.D. percentages in seropositive camels ranged between 40-372%, with a mean value of 111.86+6.14%. Toxoplasmosis, is an important zoonosis and a leading cause of abortion in animals, especially sheep and goats, worldwide. Despite its importance, however, very sparse information exists on the prevalence, epidemiology and clinical significance of toxoplasmosis in Saudi Arabian farm animals, particularly camels. This is surprising since the first record of toxoplasmosis in Saudi farm animals was a report on serological detection of *T. gondii* in indigenous dromedary camels (Hussein *et al*. (1988). Using indirect haemagglutination test (IHA), these authors detected anti-*T. gondii* antibodies in 36 (16%) out of 227 male and female camels, with higher prevalence in female compared to male, and in adults compared to young, camels. A few studies on seroprevalence of camel toxoplasmosis in the Kingdom were subsequently undertaken which revealed a wide variation depending on the study area and the test used. Thus, El-Metenawy (2000) using IHA test reported zero prevalence in 94 camels from Al-Qassim area, while Al-Anazi (2011), using latex agglutination test, reported anti-*T. gondii* antibodies in 94 (13.1%) out of 713 clinically healthy camels of either sex. In the present study, which was based on indirect ELISA tests, a higher prevalence was recorded in *Mijaheem* female camels, ranging between 26.67% to 84.06% in different regions, with an overall prevalence of 49.18%. Serological evidence of camel toxoplasmosis was also reported in other countries, with lower prevalence than that presently recorded in Saudi camels (Elamin *et al*., 1992; Sadrebazz *et al*., 2006; Hamidinegat *et al*., 2013; Gebremedhin and Tadesse, 2015). In some parts of eastern Sudan, however, the prevalence of camel toxoplasmosis was comparable to that presently recorded in Saudi camels (Jomaa *et al*., 2017) while even a higher prevalence was recorded in Maghrabian camels in Egypt, with previous history of abortion, stillbirth and increased calf mortality (Osman *et al*., 2016). Using slide toxo-agglutination test, these authors reported anti-*T. gondii* antibodies in 24 (70.6%) out of 35 camels aged < 5 years, and 8 (42.1%) out 19 camels aged less than 5 years. An association of age with incidence of toxoplasmosis was also recorded in the present study.

Statistical analyses revealed a highly significant relationship of *T. gondii* seropositivity with the location (X^2 = 53.21, p <0.0001) as well as age (X^2 = 20.62, p <0.0001), and the highest prevalence was recorded in camels aged 2-<4yr. *T. gondii* was incriminated as a cause of abortion and stillbirth in female camels and mortality in camel calves (Tibary,

2016; Osman *et al*., 2016). Apart from that, affected camels rarely exhibit clinical signs. However, Hagemoser *et al*. (1990) described a case of acute toxoplasmosis in an adult camel with history of dyspnea and accumulation of large quantity (around 24 L) of turbid fluid in its pleural cavity. Smears of the pleural fluid revealed numerous *T. gondii* tachyzoites in macrophages while high *T gondii* antibodies titers (1: 20,000) were found in pleural fluid. Manal and Majid (2008) reported diarrhea associated with congenital toxoplasmosis in camel calves in the Sudan. Using ELISA tests, they detected IgM and IgG antibodies in the sera of diarrheic and those of recovered camel calves, respectively. Riley *et al.* (2017) described a fatal case of disseminated toxoplasmosis, associated with hemorrhagic enterocolitis and hemorrhagic diarrhea, in an 11-year old dromedary camel in a zoo in USA. Histopathological examination revealed *T. gondii* cysts within lesions in the intestines, lungs and liver, while the spleen showed lymphoid depletion suggestive of immunosuppression.

Forty-nine (13.32%) out of 368 camels were positive for anti-*N. caninum* antibodies. The prevalence again varied widely in different locations, ranging from 0% in the eastern region to 38.67% in the western region. The percent O.D. values among serologically positive camels were relatively low, ranging between 40-93%, with a mean value of 42.11+2.86. As in the case of brucellosis, a highly significant association of the seroprevalence of neosporosis with location (x^2= 62.49; p <0.0001) was recorded while the association of seroprevalence with age was non-significant (x^2= 2.49; p <0.2885). *N. caninum* is another abortifacient agent that is closely related to *T. gondii* and is one of the main causes of reproductive failure in bovines worldwide (Georgieva *et al*., 2006; Wouda, 2000; Dubey and Shares, 2011). In the present study, 49 out of 368 (13.32%) camels were seropositive for neosporosis. To our knowledge, only one report of neosporosis in Saudi camels was published previously. In that report, anti-*N. caninum* antibodies were detected in the sera of 17 (4.1%) out of 412 camels using indirect fluorescent antibody test (IFAT) (Al-Anazi, 2011). The study was limited to clinically healthy adult camels in Riyadh Province and neither the sex nor breed of the camels was specified. In the present study, the serological prevalence of anti-*N. caninum* antibodies in Saudi camels was comparable to that reported in the UAE (Wernery *et al*., 2008) but higher than that reported elsewhere, namely 3.72% in Egypt (Hilali *et al*., 1998) and 3.22% - 4.16% in different parts of Iran (Sadrebazzaz *et al*., 2006; Hosseininejad *et al*., 2009). It should be pointed out, however, that these surveys were based either on IFAT or modified agglutination test (MAT) while in the present study and that in the UAE, the seroprevalence was determined using indirect ELISA, suggesting that the latter test might be more sensitive for detecting *N. caninum* antibodies than IFAT and MAT. *N. caninum* infection in farm animals is usually asymptomatic (Dubey and Shares, 2011; Elsheikha *et al.*, 2013). However, abortion is a serious consequence of neosporosis in cattle and sometimes small ruminants (Moreno *et al*., 2012). In contrast, no records are available associating *N. caninum* with reproductive problems in dromedaries. On the other hand, this protozoan was implicated as an important cause of abortion in New World camelids (Serrano-Martinez *et al*., 2007). Using immunohistochemical technique or PCR, these authors recorded *N. caninum* in 28% of aborted fetuses of llama (*Lama glama*) and alpaca (*Vicogna pacos*) in Peru.

The serological prevalence of chlamydiosis (*Cl. abortus*) in different locations ranged between 4.35% in the southern to 16% in the eastern region, with an overall seroprevalence rate of 10.05% (38/378) while the percent O.D. values among positive camels ranged between 41-143%, with a mean of 60.47+3.69. There was a significant relationship between the location with the serological prevalence of chlamydiosis (x^2 = 7.56; p <0.0227). The prevalence of chlamydiosis was also significantly associated with age (x^2 = 6.75 p <0.0342), with increased prevalence being recorded with increasing age. *Chlamydia (Chlamydophila) abortus* is also an important abortifacient agent in farm animals, especially sheep and goats. The infection is widely distributed and is believed to be responsible for 20 to 50% of all spontaneous abortions and stillbirths in sheep worldwide (Aitken, 2000; Cobb, 2009). During the present study, antibodies against *Ch. abortus* was detected in 38 (10.05%) out of 378 camels. This is the second record on chlamydiosis in Saudi camels. Previously, Hussein *et al*. (2008), using indirect ELISA, recorded anti-*Ch. abortus* antibodies in 36 (19.4%) out of 186 Saudi male and female camels, none of which exhibited clinical signs. It was also noted that seropositivity was higher by more than two folds in female versus male camels, that nearly 95% of all seropositive animals were adults aged < 4 yrs and that 70% of seropositive camels were 8 or more years old. A

similar observation was reported by Elzlitni and Elhafi (2016) in Libyan camels in which the seroprevalence of chlamydiosis was twice as high in female as compared to male camels. Few records on the serological prevalence of *Ch. abortus* in camels in countries other than Saudi Arabia are available, with prevalence rates being: 7.6% in Tunisia (Burgmeister *et al*., 1975), 11% in Egypt (Schmatz *et al*., 1978), 24% in breeding and 15% in racing camels in the UAE (Wernery and Wernery, 1990) and 12.25% in Libya (Elzlitni and Elhafi, 2016). Data on the clinical significance of chlamydiosis in camels are also very scant. Wernery and Wernery (1990) suggested that although chlamydiosis was a major cause of abortion in sheep, goats and cows, it did not seem to affect pregnancy in camels since no increase in abortion rate was observed in infected camel herds and no chlamydia was found in uterine swabs from these animals. However, Tibary (2016) stated that chlamydiosis was associated with abortion in New World camelids. Furthermore, Ali *et al*. (2012) incriminated *Ch. abortus* as a cause of ovarian hydrobursitis syndrome, which might lead to conception failure in dromedary camels, while Osman *et al*. (2016) associated chlamydiosis with reproductive failure and calf mortality in dromedaries and detected chlamydial antibodies in vaginal swabs of camels with history of abortion or stillbirth.

Finally, 158 out of 378 camels were serologically positive for *anti-C. burnetii* antibodies, giving an overall prevalence of 41.80%, while regional prevalence ranged between 23.19% in the southern region to 75.38% in the northern region. The O.D.% values ranged between 40-293%, with a mean O.D.% of 94.47+4.42. A significant association was recorded between location with prevalence, while a non-significant relationship existed between age and prevalence (x^2= 4.12; p <0.1275). Once again, only a few reports on the seroprevalence of this organism in Saudi Arabian camels are available (Hussein *et al*., 2008; Jarelnabi *et al*., 2018) in addition to preliminary studies on the detection of *C. burnetii* by PCR in clinical samples from Saudi camels (Mohammed *et al*., 2014), a comparison of *C. burnetii* prevalence in camel milk versus serum (Jar Elnabi *et al*., 2015) and the role of the camel as a major reservoir of human Q-fever in Saudi Arabia (Hussein *et al*., 2015).

The high serological prevalence of *C. burnetii* in the present camels confirms our earlier findings (Hussein *et al*., 2015; Jarelnabi *et al*., 2018). The present results are also consistent with reports from other camel rearing countries (Wernery and Kaaden, 1995), which further support the belief that these animals might serve as important reservoirs of Q-fever in camel rearing areas especially among nomads and consumers of camel meat and raw milk (Hussein *et al*., 2015). A high serological prevalence of *C. burnetii* antibodies was also reported in camels in different countries, *viz*. 66 % in Egypt (Soliman *et al*., 1992), 80% in Chad (Schelling *et al*., 2003), 71.2% and 85.3% at the individual and herd levels, respectively, in Algeria (Benaissa *et al*., 2017), 64.5% in the Sudan (Hussien *et al*., 2016) and up to 100% among nomadic camels in southeastern Ethiopia (Gumi *et al*., 2013),. Molecular studies using PCR also demonstrated *C. burnetii* shedding in feces, urine, blood and milk of seropositive Saudi camels (Mohammed *et al*., 2014). Frequent shedding of this organism is also known to occur in post-partum discharges. Using PCR, relatively high positivity of *C. burnetii* DNA was also reported in Iranian camels (Doosti *et al*., 2014).

From a clinicopathological standpoint, *C. burnetii* may cause reproductive failure in farm animals, including abortion, premature delivery, stillbirth and week offspring (APSW) complex, placentitis, retention and consequent subfertility or sterility (Agerholm, 2013; Van den Brom *et al*., 2015).

In conclusion, brucellosis, toxoplasmosis, neosporosis, chlamydiosis and coxiellosis are prevalent abortion-causing agents among indigenous *Mijaheem* camels in Saudi Arabia, with prevalence rates varying in different geographical regions and in the case of toxoplasmosis and chlamydiosis also with age. Many other factors are also known to be associated with the prevalence of these agents in farm animals, both at the individual and the farm level. These factors include geographical and climatic factors, age, sex and breed of the animal, management factors, nutrition, herd size, history of reproductive problems, mixed grazing or proximity to other farms, presence of dogs and cats and presence of ticks. These different risk factors should be investigated. Many other infectious causes of abortion may also be present in farm animals in Saudi Arabia without being recognized and studies should also be undertaken to determine their incidence in Saudi livestock. It is also important to realize that many abortion-causing organisms of animals, including those presently investigated, are of considerable public health importance

and every attempt should therefore be made to prevent their transmission from animals to humans, particularly to those individuals at greater occupational risk.

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