Serological evidence of Coxiella burnetii, Leptospira interrogans *Hardjo,* Neospora caninum *and bovine pestivirus infections in a dairy cattle herd from the United Arab Emirates*

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Summary
The serostatus of five abortigenic agents and the association between abortion history and *Coxiella burnetii* seropositivity were assessed in 350 dairy cattle from Al Ain, UAE. The bovine sera were ELISA-screened for *C. burnetii, Leptospira* Hardjo, *Neospora caninum*, and *Brucella abortus* antibodies, plus bovine pestivirus (BVDV) antigen. The serology data were collated and the level of significance between the proportions of *C. burnetii*-seropositive cattle with and without abortion history assessed by the Z test of two proportions. Of the 350 cattle, 41.4%, 1.7%, 1.4%, 0.3%, and 0.0% were seropositive to the above pathogens, respectively. Besides, 61.9%, 2.9%, 1.0%, 0.0%, and 0.0% of the 105 cattle with history of abortion and 32.7%, 1.2%, 1.6%, 0.0% and 0.0% of the 245 seropositive cattle with no history of abortion were also seropositive for the above pathogens respectively. Moreover, the proportion of *C. burnetii*-seropositive cattle with history of abortion were significantly higher than the *C. burnetii*-seropositive ones without abortion history (p-value < 0.01). Apparent *C. burnetii* infections were relatively higher than the other four pathogens suggesting this bacterium contributed to abortion in the herd. Additional research on the public and bovine health implications of *C. burnetii* and *Leptospira* in the UAE are urgently needed.

Introduction

Bovine abortion is widely recognized as a cause of significant economic losses in dairy cattle worldwide (Knudtson and Kirkbride 1992, Thurmond *et al.* 1990). Even though non-infectious factors may cause reproductive failure, abortigenic infectious agents are likely to cause more epidemiologically dynamic forms of abortion in dairy cattle (Kaveh *et al.* 2017, Knudtson and Kirkbride 1992). Universally recognized abortigenic bacterial pathogens of cattle include, but are not limited, to *Brucella abortus* (Okumu *et al.* 2019, Shabbir *et al.* 2011), *Coxiella burnetii* (Bildfell *et al.* 2000, Cabassi *et al.* 2006), *Campylobacter foetus* (Michi *et al.* 2016), *Leptospira* spp. (Delooz *et al.* 2018) and *Listeria monocytogenes* (Yağcı-Yücel *et al.* 2014). In addition, a few abortigenic protozoans like *Neospora caninum* (Okumu *et al.* 2019, Shabbir *et al.* 2011, Yildiz *et al.* 2017), *Trichomonas foetus* (Michi *et al.* 2016), *Toxoplasma gondii* (Pagmadulam *et al.* 2018), and *Sarcocystis* spp. (Rassouli *et al.* 2014) have also been reported. Similarly, a number of abortigenic viruses like bovine viral diarrhoea virus (BVDV) (Aslan *et al.* 2015, Asmare *et al.* 2018) and bovine herpesvirus-1 (Chastant-Maillard, 2015) have also been reported. Periodically, a number of abortigenic vectorborne viruses like Rift Valley fever virus (Ali *et al.* 2012), Bluetongue virus (Ali *et al.* 2012, Nusinovici *et al.* 2012), and Akabane virus (Kirkland 2015) have been particularly reported in tropical and subtropical regions of the world. Finally yet importantly, a number of abortigenic fungal pathogens including, but not limited to, *Aspergillus* sp. and *Mortierella wolfii* have also been reported (McCausland *et al.* 1987).

Despite anecdotal reports on incidents of abortion problems at a number of dairy farms in the United Arab Emirates (UAE), there is complete absence of country-specific peer-reviewed literature on bovine abortion. Such paucity of UAE-specific literature is a hindrance to development of evidence-based methods for the control and management of reproductive failure in dairy cattle herds in the country. It is noteworthy that biosecurity guidelines exist for the control of brucellosis, coxiellosis, and leptospirosis in the UAE but not for neosporosis or BVD (ADAFSA 2011). While such biosecurity guidelines exist for the former three diseases, however, only brucellosis has previously had an active surveillance program. The broader aim of the present pilot study was to collect baseline data on the serostatus of selected abortigenic pathogens of dairy cattle in the Al Ain region, UAE. Specific study objectives were to determine the serostatus of *C. burnetii*, *B. abortus*, *L.* Hardjo, *N. caninum*, and BVDV in an intensively managed dairy cattle herd from the periurban dairy production system of Al Ain region, UAE. The study also evaluated the association between the *C. burnetii*-seropositivity and history of abortion.

Materials and methods

Study dairy farm and sample size calculation

Blood samples were collected by venipuncture from 350 randomly selected dairy cattle that belonged to a herd of 6,000 Holstein-Friesian cattle. The study farm is located in the Al Ain region, Emirate of Abu Dhabi, UAE and up until the time of the research, the dairy farm had been experiencing abortion problems. The sample size was determined using the formula:

$$n = z_{\alpha}^2 pq/L^2$$

where, n = sample size, z_{α} = normal deviate (1.96) at 5% level of significance, p = estimated prevalence, q = 1 – p and L = precision of estimate usually at 5% (Thrusfield 2007).

For the sample size calculation, *a priori* bovine coxiellosis prevalence of 22.3% reported in Iran was used (Azizzadeh *et al.* 2012).

The sample size was therefore derived as follows:

$$n = (1.96)^2 \times 0.223 \times \frac{1 - 0.223}{(0.05)^2} = \approx 266$$

To adjust for potential non-compliance and design effect, the calculated sample size was increased to 350. Following collection, the blood samples were allowed to clot at room temperature (rT), the sera separated by centrifugation at 4,000 rpm, 5 min and then kept at - 20 °C until testing.

Immunoenzymatic assays

The screening for *C. burnetii* antibodies was done using an indirect *C. burnetii* ELISA (Q fever *C. burnetii* antibody test kit, IDEXX Laboratories, Switzerland) according to the manufacturer's instructions. After diluting test sera and positive and negative controls to 1:400 in the kit wash buffer solution, 100 µL/well of each were dispensed into 96 microtiter plate wells pre-coated with inactivated *C. burnetii* antigen. After incubating the plates at 37 °C for 60 min, they were washed three times with the kit wash buffer, 100 µL/well of a peroxidase labelled anti-ruminant IgG conjugate added and the plates then incubated for 60 min at 37 °C. The plates were then washed three times with the kit wash buffer like before and 100 µL/well of the 3,3',5,5'-tetramethylbenzidine (TMB) substrate added. After stopping the reaction, the ELISA plates were read at rT in a spectrophotometer (Bio Tek Instruments. Inc. Highland Park, USA) at 450 nm and the results expressed as a percentage of the ratio of the test sample OD_{450} to the positive control OD_{450} (S/P%). In line with recommendations of the test kit manufacturer, the test samples with S/P% ≥ 40% were interpreted as positive while those with S/P% < 30% were deemed negative.

For the indirect ELISA for *B. abortus* antibodies (*Brucella abortus* antibody test kit, IDEXX Laboratories, Switzerland), 100 µL/well of test sera, as well as the positive and negative controls, were diluted 1:10 in the kit sample diluent, placed in the 96 microtitre wells and the plates tightly sealed. After incubating at 37 °C for 60 min, the plates were washed three times using 300 µL/well of the kit wash solution and 100 µL/well of peroxidase labeled anti-ruminant IgG conjugate added. After tightly sealing the plates, they were further incubated at 37 °C for 60 min. Following the incubation step, the plates were washed three times with 300 µL/well of the kit wash buffer and 100 µL/well of TMB substrate then added. After stopping the reaction, the plates were read at 450 nm using a spectrophotometer (Bio Tek Instruments. Inc. Highland Park, USA). In line with the test kit manufacturer recommendations, samples having S/P% < 80% were deemed as negative while those with S/P% values ≥ 80% were considered as positive.

The screening of test sera for *L.* Hardjo antibodies was done using indirect ELISA (*Leptospira* Hardjo Ab bovine ELISA, Demeditec Diagnostics, Germany) according to manufacturer's instructions. After washing the plates five times with 100 µL/well of the kit wash buffer, 100 µL/well of test sera diluted at 1:100 in the kit sample diluent as well as the

negative and positive controls diluted at 1:50, were separately dispensed into the microtiter plates coated with *Leptospira* antigen. The plates were incubated at 37 °C for 60 min and then washed five times with the kit wash buffer. Next, 100 µL/well of horseradish peroxidase labelled anti-bovine IgG conjugate were added, and the plates incubated for another 60 min at 37 °C. After five washes, 100 µL/well of TMB substrate were added and the plates incubated at rT in darkness for 15 min. After stopping the reaction, the plates were read at 450 nm in a spectrophotometer (Bio Tek Instruments, Inc. USA). In line with recommendations of the test kit manufacturer, test samples with S/P% ≥ 34% were considered as positive for *L*. Hardjo antibodies while samples with S/P% < 34% deemed as negative.

The screening for *N. caninum* antibodies was done using an indirect ELISA (*Neospora caninum* Antibody Test Kit, IDEXX Laboratories, USA) following the kit manufacturer's instructions. Briefly, 100 µL/well of test samples diluted at 1:100 in the kit sample diluent as well as undiluted negative and positive controls were dispensed into microtiter plates coated with *N. caninum* antigen. The plates were then incubated at rT for 30 min after which they were washed four times with kit wash buffer. 100 µL/well of peroxidase labelled anti-ruminant IgG conjugate were added and the plates incubated at rT for 30 min. The plates were then washed four times with kit wash buffer, 100 µL/well of TMB substrate added to each well, and the plates further incubated at rT in darkness for 15 min. After stopping the reaction, the plates were read using a spectrophotometer (Bio Tek Instruments. Inc. Highland park, USA) at 630 nm. In line with the test kit manufacturer recommendations, the test samples with S/P% ≥ 50% were considered as positive while those with S/P% < 50% were deemed as negative.

The detection of BVDV antigen was done using an antigen capture ELISA (Bovine Viral Diarrhoea Virus antigen test kit/serum plus, IDEXX Laboratories, Switzerland) according to the manufacturer's kit instructions. Briefly, 50 µL of the detection antibodies were dispensed into a microtiter plate wells pre-coated with monoclonal antibodies specific for BVDV antigen. This was followed by 50 µL of the test sera alongside the negative and positive controls. The plates were then tightly sealed and incubated at 37 °C for 2 h. The plates were then washed five times with the kit wash buffer. After incubating at rT with 100 µL/well of peroxidase labelled anti-ruminant IgG conjugate for 30 min, the plates were washed five times with the kit wash buffer, 100 µL/well of TMB substrate were then added to each well and the plates incubated at rT for 10 min. After stopping the reaction, the plates were read at 450 nm using spectrophotometer (Bio Tek Instruments. Inc. Highland park, USA). The results were expressed as corrected OD values (S-N) for each sample using negative control. In line with the test kit manufacturer's recommendations, the test samples with (S-N) ≤ 0.300 were considered as negative, while those with (S-N) ≥ 0.300 were considered as positive.

Statistical data analysis

The serology data were graphically presented and descriptive statistics done to demonstrate the proportions of cattle that were seropositive to the five abortigenic agents. In addition, the Z score test for two proportions was applied to evaluate the level of significance in the difference between the proportion of *C. burnetii*-seropositive cattle that had a history of abortion and the proportion of *C. burnetii*-seropositive cattle that did not have a history of abortion (p value 0.01).

Refer to the formula below relating to the test hypothesis:

Null hypothesis $H_0: p_1 - p_2 = 0$

Alternative hypothesis $H_1: p_1 - p_2 > 0$

$$\frac{(\bar{p}_1 - \bar{p}_2) - 0}{\sqrt{\bar{p}(1-\bar{p})\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

Owing to the few samples that were seropositive for *B. abortus*, *L*. Hardjo, *N. caninum*, and BVDV, this test was not performed for these agents.

Results

Descriptive statistics and data analysis

Of the 350 bovine serum samples that were tested by ELISA, 41.4% (145/350) were seropositive for *C. burnetii*, 0.0% (0/350) for *B. abortus*, 1.7% (6/350) for *L*. Hardjo, 1.4% (5/350) for *N. caninum*, and 0.3% (1/350) for BVDV (Figure 1). By comparison 58.6% (205/350) were seronegative for *C. burnetii*, 100.0% (350/350) were seronegative for *B. abortus*, 98.3% (344/350) seronegative to *L*. Hardjo, 98.6% (345/350) negative to *N. caninum*, and 99.7% (349/350) were negative for BVDV antigen (Figure 1). Furthermore, of the dairy cattle that were screened by ELISA in the present study, 30.0% (105/350) had history of abortion while 70.0% (245/350) did not. Interestingly, of the 105 cattle that had history of abortion, 61.9% (65/105) were seropositive for *C. burnetii*, 2.9% (3/105) for *L*. Hardjo, 1.0% (1/105) for *N. caninum*, 0.0% (0/105) for BVDV, and 0.0% (0/105) for *B. abortus*. When the number of seropositive cattle without history of abortion are considered, 32.7% (80/245) were positive for antibodies against *C. burnetii*, 0.0%

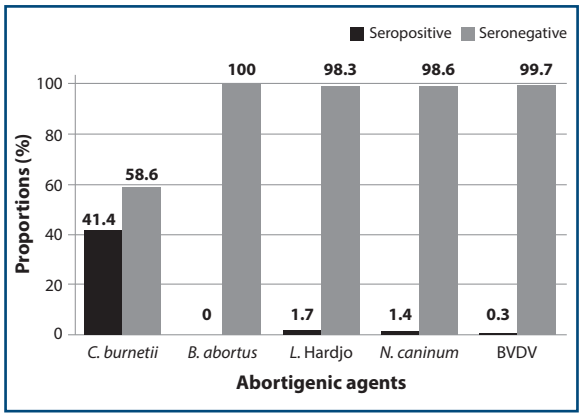

Figure 1. *Bar graph showing the comparative proportions of cattle that were seropositive and seronegative for five abortigenic agents including* Coxiella burnetii, Brucella abortus, Leptospira *Hardjo,* Neospora caninum*, and bovine virus diarrhoea virus (BVDV).*

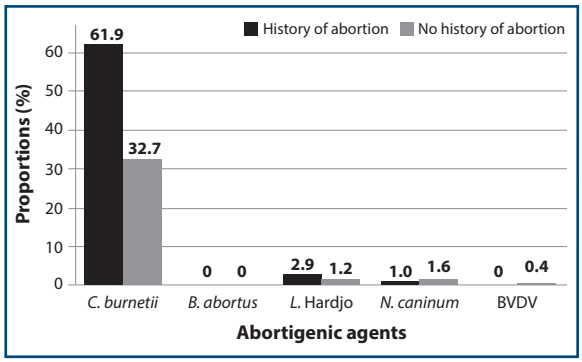

Figure 2. *Bar graph showing the comparative proportions of dairy cattle with or without history of abortion that were seropositive for five abortigenic agents including* Coxiella burnetii, Brucella abortus, Leptospira *Hardjo,* Neospora caninum*, and bovine virus diarrhoea virus (BVDV).*

(0/245) for *B. abortus*, 1.2% (3/245) for *L.* Hardjo, 1.6% (4/245) for *N. caninum*, while 0.0% (0/245) were negative for BVDV antigen (Figure 2). The z-test shows that the proportion of *C. burnetii* seropositive cattle with a history of abortion was significantly higher than the *C. burnetii* seropositive cattle that did not (p-value < 0.01) with the difference between the proportions of 29.3% (95% CI: 18.2% to 40.2%).

Discussion and conclusions

The present pilot study evaluated the serostatus of five abortifacient pathogens in an intensively managed dairy cattle herd from Al Ain, UAE. As the study herd had a perennial history of abortion, and since the proportion of seropositive animals was only high for *C. burnetii*, the present survey further assessed if history of abortion was significantly associated with being seropositive for this pathogen. According to the present data, the dairy cattle herd under study demonstrated variable serostatus in respect to the five abortigenic agents. To the author's knowledge, this is the first time serological evidence is adduced on apparent *C. burnetii*, *L.* Hardjo, *N. caninum*, and BVDV infections in dairy cattle in the UAE. As anecdotal reports have previously suggested high prevalence of animal brucellosis, it was rather unexpected that all the 350-screened cattle were seronegative to *B. abortus* antibodies. It should be noted that biosecurity guidelines exist for the control of brucellosis (ADAFSA 2011), and the surveillance interventions for the disease have previously been instituted in the country (M.E.H Mohamed, personal communication). It is possible that the biosecurity measures adopted in response to previous concerns over animal brucellosis may have been effective.

While *C. burnetii* antibodies have previously been reported in a number of animal species on UAE territory (Afzal *et al.* 1994, Chaber *et al.* 2012, Hassan *et al.* 2018, Lloyd *et al.* 2010), this is the first time research data suggests coxiellosis infection in dairy cattle. As serological evidence of *C. burnetii* infection was previously reported in racing dromedary camels (Afzal *et al.* 1994), a dama gazelle that had aborted (Lloyd *et al.* 2010), as well as semi-free ranging wild ungulates (Chaber *et al.* 2012), and sheep and goats (Hassan *et al.* 2018), a complex coxiellosis epidemiology that arguably involves cross-species *C. burnetii* transmission cannot be ruled out. This can only be clarified through undertaking more comprehensive epidemiological studies in the country. It is noteworthy that of the five abortigenic agents evaluated in the present pilot study, the proportion of *C. burnetii*-seropositive cattle was comparatively greater than for the other four agents for which the proportions of seropositive cattle were 0.0%, 0.3%, 1.4%, and 1.7% for *B. abortus*, BVDV, *N. caninum*, and *L.* Hardjo, respectively. To further evaluate the abortigenic significance of *C. burnetii*, the Z test was applied to the proportion of *C. burnetii*-seropositive cattle that had history of abortion and seropositive animals that did not have such a history. As it turned out, the data demonstrated there was statistically significant difference between the two groups (Z test of two proportions; p < 0.01) further implicating *C. burnetii* causing abortion in the affected herd. Indeed, while the list of abortigenic agents screened for was not exhaustive, future study protocols will need to further delineate the role of *C. burnetii* in bovine abortions in the study region of Al Ain and beyond. Elsewhere, detection of *C. burnetii* in the foetal membranes and other biological specimens taken from aborted or stillborn foetuses (Agerholm 2013, Muskens *et al.* 2012) has been reported. Moreover, *C. burnetii*-induced placentitis was demonstrated in aborting cattle (Bildfell *et al.* 2000; Cabassi *et al.* 2006).

It should be noted that while biosecurity guidelines exist for the control of coxiellosis (ADAFSA 2011), no surveillance interventions for the disease exist in the country and as such this gap needs to be urgently addressed. It should be noted that the proportions of *L.* Hardjo, *N. caninum* and BVDV-seropositive cattle were comparatively lower than for *C. burnetii*. As the former three organisms are primary abortigenic agents of cattle (Asmare *et al.* 2018, Delooz *et al.* 2018, Yildiz *et al.* 2017), their animal health implications in cattle in the UAE needs to be further investigated. This is more so important since this is the first time they are being reported in the country. Further still, as *Leptospira* is also an important zoonotic pathogen (Garshasbi *et al.* 2018), the potential public health significance of this bacterium should also be evaluated in the UAE. It should be noted that biosecurity guidelines exist for the control of leptosiprosis (ADAFSA 2011), even when no surveillance program for the disease exists in the country. After additional leptospirosis research, this gap needs to be addressed.

In summary, the present data warrant additional comprehensive research into bovine abortion in the UAE. It should be noted that while biosecurity guidelines exist for the control of brucellosis, coxiellosis, and leptospirosis in the UAE (ADAFSA 2011) there are currently no active surveillance programs for these diseases. Considering the present data therefore, initiating an active surveillance program for coxiellosis while further investigating the epidemiology of all the studied diseases is strongly warranted. In particular, further research focused on delineating the animal and public health implications of *C. burnetii* infection is recommended. By continuing to generate baseline data on infectious causes of bovine abortion, such studies will, in future, ultimately pave way to more evidence-based disease control and management strategies for bovine abortion in the country. In the meantime, the present data should inform diagnostic investigation protocols whenever investigating cases of bovine abortion in the country.

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