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

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Abstract

To date, no epidemiological studies have evaluated bovine abortion in the UAE. Therefore, the present study evaluated the serostatus of five abortigenic agents in a dairy cattle herd from Al Ain region, UAE. Additionally, the association of abortion history with Coxiella burnetii seropositivity was also evaluated. Indirect ELISA screened 350 sera from dairy cattle for C. burnetii, Leptospira Hardjo, Neospora caninum, and Brucella abortus antibodies while a sandwich ELISA tested the samples for bovine virus diarrhoea virus (BVDV) antigen. The serological data were summarized by descriptive statistics and the Z test of two proportions applied to assess the statistical significance between the proportions of C. burnetii-seropositive cattle with and without abortion history. Of the 350 cattle, 41.4%, 1.7%, 1.4%, 0.3%, and 0.0% were seropositive to C. burnetii, L. Hardjo, N. caninum, BVDV, and B. abortus respectively. Furthermore, of the 105 cattle with history of abortion, 61.9%, 2.9%, 1.0%, 0.0%, and 0.0% were seropositive to these pathogens respectively. Conversely, of the 245 seropositive cattle with no history of abortion 32.7%, 1.2%, 1.6%, 0.0% and 0.0% were seropositive to these pathogens respectively. Further still, the z-test showed the proportion of C. burnetii seropositive cattle with a history of abortion to be significantly higher than the C. burnetii seropositive animals without abortion history with a difference between the proportions of 29.3% [p-value < 0.01; 95% CI: 18.2% to 40.2%]. Compared to the other four pathogens, the C. burnetii infection level in the dairy herd was relatively higher. As C. burnetii is abortigenic, it may have causally contributed to reproductive failure in the cattle herd. Additional epidemiological studies are needed to further elucidate the abortigenic significance of C. burnetii and the other four pathogens in the national dairy cattle herd. Besides, the public health implications of C. burnetii and Leptospira need to be evaluated.

Introduction

Bovine abortion is widely recognized as a cause of significant economic losses in dairy cattle worldwide (Knudtson & 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 & 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),*Leptospir* a 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 virus 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 reported particularly 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 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. 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. burnetti* ,*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.

Material 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 6000 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, $n = z_{\alpha}^2 pq/L^2$ (Thrusfield, 2007, 3rd Edition, pp. 283-240): 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%. For the sample size calculation, a priori bovine coxiellosis prevalence of 22.3% reported in Iran (Azizzadeh*et al.* 2012).

The sample size was therefore derived as follows, n:

$n = (1.96)^2 \times 0.223 \times \frac{1-0.223}{(0.05)^2} = \approx 311$

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 4000 rpm, 5 min and then kept at$-20C^{\circ}$ 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), *B. abortus* , *L. Hardjo* , *N. caninum* , and by BVDV antigen capture ELISA according to the kit 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 addedm 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 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 then expressed as a percentage of the ratio of the test sample OD_{450} to the positive control OD_{450} (S/P %). The test samples with S/P % [?] 40% were interpreted to be positive while those with S/P% $<$ 30% were deemed negative.

For the indirect ELISA for *B. abortus* antibodies, 100 µL/well of test sera, as well as the positive and negative controls, were diluted 1:10 in the kit sample diluent were 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 3,3',5,5'-tetramethylbenzidine or TMB substrate then added. After stopping the reaction, the plates read at 450 nm

using a spectrophotometer (Bio Tek Instruments. Inc. Highland Park, USA). Samples having S/P % < 80 % were deemed negative while those with S/P % values [?] 80 % were determined to be 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 then incubated at 370C for 60 min after which they were washed five times with kit wash buffer, 100 µL/well of horse radish peroxidase labelled anti-bovine IgG conjugate added, and the plates incubated for another 60 min at 370 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). Test samples with S/P % [?] 34% were considered positive for*L. Hardjo* antibodies while samples with S/P% < 34% deemed 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 and then 100 µL/well of peroxidase labelled anti-ruminant IgG conjugate 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. Test samples with S/P % [?] 50% were determined to be positive while those with S/P% <50% were deemed 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 370C 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 after which 100 µL/well of TMB substrate were 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. Test samples with (S-N) [?] 0.300 were considered negative, while test samples with (S-N) [?] 0.300 were considered positive.

Statistical data analysis

The serology data were tabulated, 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; H0: p1 - p2 = 0; Alternative hypothesis H1: p1 - p2 > 0

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

Owing to the few samples that were seropositive for *B. abortus* ,*L. Hardjo* , *N. caninum,* and BVDV, this test was not 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 (Table 1; 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 (Table 1; 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 have any history of abortion. 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% (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 (Table 2; Figure 2). It is noteworthy that of the 105 cattle that had a history of abortion, 61.9% (65/105) were seropositive for *C. burnetii* . By comparison, of the 245 cattle that did not have a history of abortion, 32.7% (80/245) were seropositive for*C. burnetii* . 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 have a history of abortion [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. As the present data have shown, the dairy cattle herd that was the subject of the study demonstrated variable serostatus in respect to the five study 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. While anecdotal reports have previously suggested high prevalence of animal brucellosis, it was rather an unexpected finding that all the 350-screened cattle were seronegative to *B. abortus*antibodies. It is possible that biosecurity measures adopted in response to previous concerns of animal brucellosis in the UAE 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 by 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 agent for which the proportions of seropositive cattle ranged from 0.0%, through to 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. burnetti* , the Z test of two proportions 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* -in causation of abortion in the affected herd. Indeed, while the list of abortigenic agents screened for was not exhaustive, future study protocols will need to further help delineate the role of *C. burnetii* in bovine abortions in the study region of Al Ain and beyond. Elsewhere, detection of *C. burnetti* 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* was demonstrated in aborting cattle placentitis (Bildfell *et al.* 2000; Cabassi *et al.* 2006). 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 *Leptospir* a 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.

In summary, the present data warrant additional comprehensive research into bovine abortion in the UAE. In particular, further research focused on delineating the animal and public health implications of *C. burnetii* infection is urgently needed. 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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Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

Conflict of Interest Statement

All the listed authors have contributed to this manuscript and declare that there are no financial or other forms conflict of interest that would have influenced their objectivity towards contribution to publication of this work.

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TABLE AND FIGURE LEGENDS

Table 1.

Summary of serological data showing proportions of cattle that were seropositive and those that were seronegative to the five study abortigenic agents.

Table 2.

Proportions of seropositive dairy cattle that had history of abortion compared to the seropositive cattle that did not have history of abortion.

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.

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