Pathogens Affecting the Reproductive System of Camels in the United Arab Emirates

with emphasis on Brucella abortus, Bovine Viral Diarrhoea Virus and Bovine Herpes Virus-1: a serological survey in the Al-Ain region

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Abstract

Brucella species, BVDV and BHV-1 are important worldwide pathogens that primarily infect cattle and other ruminants and are associated with serious economic impact to the animal production sector. Camels are indispensable domesticated animals for the people of desert areas, such as the Arabian Peninsula, and share the same environment and pasture with cattle, goats, sheep and other ruminants, which increase the likelihood of exposure to these pathogens. The investigation presented in this thesis was a serological survey conducted in Al-Ain region of the United Arab Emirates (UAE), and was designed to determine the presence of antibodies against *Brucella* species, BVDV and BHV-1. Serum samples from 812 camels of both sexes, and different ages were collected from the local central abattoir, the camel market and 10 private farms. A commercial competitive ELISA was used to determine the presence of *Brucella abortus* antibodies, and a blocking ELISA, was used for the detection of antibodies to BVDV and BHV-1.

Samples with suspected or non-specific positive results, were retested with confirmatory tests such as Complement Fixation and Virus Neutralization test, as required. No sera from the 812 camels in this study tested positive for any of the three pathogens.

Without underestimating the progress and efforts of the local veterinary authorities, these results indicate the need for more extensive sampling and comprehensive surveys on a systematic epidemiological basis and including other pathogens. It is also necessary to reconsider the criteria for performance and validation of the assays used. This will lead to a better understanding of the pathogenesis of the agents involved, and consequently enhance knowledge to improve existing control and eradication programmes of infectious diseases in camels.

Keywords: camels, C-ELISA, B-ELISA, Brucella abortus, BVDV, BHV-1. United Arab Emirates

CONTENTS

Abbreviations

B. abortus	*Brucella abortus*
BHV-1	Bovine herpesvirus-1
BVDV	Bovine viral diarrhoea virus
ELISA	Enzyme-linked immunosorbent assay
B-ELISA	Blocking ELISA
C- ELISA	Competitive ELISA
cp	Cytopathic
CPE	Cytopathic effect
CFT	Complement fixation test
IBP	Infectious balanoposthitis
IBR	Infectious bovine rhinotracheitis
IPV	Infectious pustular vulvovaginitis
IPX	Immuno peroxidase
mABs	Monoclonal antibodies
MD	Mucosal disease
ncp	Non-cytopathic
NWC	New world camels
OD	Optical density
OWC	Old world camels
PCR	Polymerase chain reaction
PI	Persistently infected
RNA	Ribonucleic acid
S-LPS	Smooth lipopolysaccaride
RT-PCR	Reverse transcriptase polymerase chain reaction
UAE	United Arab Emirates
VI	Virus isolation
VNT	Virus neutralization test

Background

The United Arab Emirates is located in the northern-eastern corner of the Arabian Peninsula, boarded by the Arabian Sea, Saudi Arabia, Qatar and Oman. Approximately, 65% of the total country area is occupied by desert, a suitable habitat for camel species.

Map of the United Arab Emirates

Source: http://www.lib.utexas.edu/

Single humped camels (*Camelus Dromedarius*) and the two humped camels (*Camelus Bactrianus*) are defined as Old World Camels (OWC), whereas the camelids from South America (llamas, alpacas, vicuñas and guanacos) are defined as the New World Camels (NWC). In the Arab Gulf states, dromedaries re an essential source of human food, transportation and entertainment. Improvements in management methods and provision of efficient veterinary services have effectively preserved the number of camels and consequently their potential as an additional source of wool, milk and meat (reviewed by Wernery & Kaaden 1995).

Camels have been endowed with particular anatomical and physiological traits, and a relatively low susceptibility to a wide range of diseases (Wernery & Kaaden, 1995). This has provided them with endurance for survival and to undertake difficult tasks: they are the most suitable pack animal for multipurpose use in the harsh climate prevailing in the region. Ownership of healthy camel herds offers a remarkable social reputation and dignity (Kumar, 2001).

The uniqueness of camel reproductive characteristics are evident in their epithelio-chorial diffused placenta, and the exclusive location of pregnancy at the 8-15 cm long left horn of its Tshaped bicornate uterus (Kuhad *et al.,* 2001). The uterus has a body of smooth endometrium 2-3 cm in length. The ovaries are flattened lobulated structures, enclosed in conical-shaped ovarian bursa, with apexes leading to the oviducts. Camels are induced ovulators, and ovarian activity

is mainly follicular. The *Corpora lutae* is present exclusively during the 14-month long pregnancy. Single foetus pregnancy is the rule, with twin pregnancy a rare event (Musa & Abu Sineina, 1976; Kumar *et al*., 2001; Skidmore, 2002).

Due to their confinement in remote areas far from urban centres, the camel's susceptibility to a number of bacterial and viral diseases infecting other ruminants e.g. tuberculosis, Foot and Mouth disease (FMD), and abortion causing pathogens e.g. *Brucella*, Bovine Viral Diarrhoea Virus (BVDV) and Bovine Herpes Virus-1 (BHV-1), was minimal, (Bitter, 1986). Socio-economical developments have imposed a great shift to pastoralist life-style and brought them and their camels nearer to urban areas (Hjort, 1988). Reproductive failure in camels has been reported in the region, and *Toxoplasma gondii*, *Chlamydia spp*. and leptospira-like organisms have been isolated (Wernery & Kaaden, 1995). Uterine smears and biopsies, as well as serology, represent an invaluable source for studying genital infections in camels that result in reduced fertility or foetal death with or without abortion (Wernery & Kaaden, 1995). The scarcity of essential and reliable data on the prevalence of *Brucella abortus*, BVDVand BHV-1 in camels has been a major hindrance for establishing appropriate schemes for control of these diseases.

Study of literature

1. Brucella abortus

Epidemiology and pathogenesis

The members of *Brucella* genus are the etiological agents of brucellosis, which is a worldwide important zoonotic disease, except in developed countries where national eradication plans have been successfully adopted. *Brucella spp* are pathogens that directly cause heavy economic losses to the animal production industry due to abortion, delayed or persistent infertility, drop in milk production, and culling of infected animals (For review see: Nielsen & Duncan 1990).
Brucellosis is geographically widespread infection. Prevalence figures from various countries are presented in Table 1.
Brucella spp are obligate parasites requiring reservoirs for maintenance, which is clearly noticed in the susceptible broad host spectrum. However, major *Brucella spp*. exhibit host preference, such as: *B. abortus* (cattle), *B. melitensis* (goats), *B. ovis* (sheep), *B. suis* (pigs), *B. canis* (dog), *B. neotome* (desert or wood rats), and *B. maris* (marine mammals). In addition, the organism establishes chronic infections in game species and wild ruminants that increase the spreading potential of the disease. Brucellosis is of public health concern., as humans are considered the end hosts of *B. abortus* associated with a moderately severe infection, known as "undulant fever", and *B. melitensis*, *B. canis* and *B. suis* cause the much more severe disease known as "Malta fever" (For review see Quinn *et al*., 2002).

Several physical and biochemical characteristics such as colonial morphology, metabolic patterns, culture requirements, and dyes and phages susceptibility are used for the identification and biotyping of species and biovars. *Brucella abortus* has been isolated from at least nine species of domestic animals, and has nine

biotypes, although another 22 are reported, which indicates a degree of genetic plasticity (Farrell & Robertson, 1967; Salem, 1987).

Brucella spp. are small gram negative, non-motile, non-spore-forming facultative intracellular coccobacilli. They are also aerobic, carboxyphylic, non-haemolytic, catalase, urease and oxidase-positive, and non-fermenters of sugar. Virulent strains form smooth colonies and have different surface antigens. The loss of virulence is associated with the transformation of smooth colonies into rough ones (Roope *et al.,* 1991;Carter *et al.,* 1995; Carter *et al*., 2004).

Cattle of all ages are susceptible to *B. abortus*, however, sexually mature animals and pregnant dairy cattle are most commonly affected, compared to young animals that acquire the pathogen either *in utero* or postnatally (Fensterbank, 1978; Crawford *et al.,* 1986; Ray *et al.,* 1988).

The reticulo-endothelial system and the reproductive tract are sites of predilection for *B. abortus.* During late gestation, the disease is characterized by abortion in non-vaccinated heifers. Orchitis, epididymitis, seminal vasculitis, with impaired fertility, are the usual clinical observations in bulls. *B. abortus* infection occurs in the oral mucosa or naso-pharynx through ingestion or inhalation: occasionally infection is through the conjunctivae or genitalia. From the portal of entry, the pathogen is transported either free or within the phagocytes to regional lymph nodes, and is then followed by augmented replication in the macrophages and haematogenous dissemination (Ko & Splitter, 2003). The marked capacity for intracellular survival is crucial for the pathogenicity of the microorganism: *Brucella spp* interfere with phagocyte function, avoid the intraphagocytic killing mechanisms, and are protected from the immune system, humoral and cellular bactericidal mechanisms. Consequently, an extensive localization of the bacteria occurs in bone marrow, mammary glands and sexual organs accompanied by formation of granulomatous foci that may abscess. After abortion the udder may persistently harbour the infectious bacteria, generating a focus for udder-originated bacteraemia that repeatedly invades the gravid uterus in each following pregnancy (for review see Radostits & Arundel, 2000)

The pathogenesis of the disease is governed by the degree of virulence, the magnitude of the infecting dose, and host susceptibility. The introduction of animals of unknown origin and disease status into a disease-free herd is associated with inter-herd transmission; whereas, the intra-herd spread is facilitated by horizontal as well as vertical transmission (For review see: Crawford *et al.,* 1990; Price *et al.,* 1990).

Camels are considered susceptible to *B. abortus* infection, especially when they are in contact with infected large or small ruminants. The incidence and prevalence of brucellosis in camels has been clearly related to breeding and husbandry practices; therefore, a low prevalence has been associated with nomadic or extensively kept pastoralist camels, and a higher prevalence has been associated with intensive or semi-intensive management systems (Richard, 1980; Abbas & Agab, 2002*).*

Diagnosis

Accurate diagnosis of brucellosis is achieved by laboratory methods, as abortion, the sole suggestive clinical sign, is not pathognomonic. The detection of the microorganism is by direct examination of the clinical specimens through conventional staining or immunohistochemistry (IHC), followed by isolation and identification of the microorganism (For review see: Carter *et al*., 1995). Aborted foetuses, placenta, uterine exudates, testicular and epididymal abscesses, which contain appreciable quantities of the infectious brucella, are materials recommended for laboratory diagnosis. When positive cultures are not observed, serological tests are essential for a presumptive diagnosis (Nielsen & Duncan, 1988). Serological procedures are used for detecting the presence of antibodies in blood, milk, whey, vaginal mucus, seminal plasma or muscle extracts. Numerous tests with varying degrees of specificity and sensitivity are available, but many are no longer in use. The plate or tube serum agglutination tests (SAT), Rose Bengal plate test (RBPT), buffered plate antigen (BPAT) or card test, and the Milk Ring Test (MRT) are classic sensitive screening tests. The highly specific mercaptoethanol (2-MER), complement fixation tests, and different ELISA formats are described as supplementary and confirmatory, although part of the tests are also applicable for screening purposes (Angus & Barton, 1984; Radostits& Arundel, 2000).

The Complement Fixation Test (CFT)

The CFT is of the highest specificity and is considered as a definitive diagnostic test after organism isolation. Sheep red blood cells, rabbit anti-sheep sera, and guinea pig serum, as sources of active complement, are the basic reagents employed. False negative reaction occurs with a single dilution or the presence of excess IgG2 and is associated with the so-called, pro-zoning phenomenon. Another drawback is the inefficiency in detecting 2% of culture positive animals at abortion. However, automation of the test has provided a practical solution to the technical difficulties associated with the test (For Review see Nielsen and Duncan, 1990).

Enzyme Linked Immunosorbent Assay (ELISA)

The continuous efforts to improve serological procedural systems have resulted in the wide acceptance of ELISA. The indirect ELISA, for example, detects all isotypes of antibodies, but lacks the efficiency for differentiation between post-vaccine antibodies from those induced by natural infections or cross-reacting bacterial antigens (Nielsen *et al.*, 1996a; 1996b); thus, it is well suited for serological surveys where vaccination is not practiced (Samartino *et al*., 1999). The antigen used can be the complete bacterial cell or semi to highly purified smooth lipopolysacharide (S-LPS). However, less purified antigens have the advantage of reduced micelle formation and are more stable than the highly purified forms. In addition, the selection of anti IgG1 heavy and light chain reagents e.g. mouse anti-bovine IgG1, increase specificity and reduce false positive reactions (Nielsen & Duncan, 1990).

Competitive ELISA (C-ELISA) using monoclonal antibodies was pioneered by Quinn *et al.* (1984), and is based on competition between two antibody populations for a limited number of antigenic epitopes. Conjugated monoclonal antibodies to a single epitope, with highly purified antigens, guarantee the specificity of the test. If *B. abortus* antibodies are present in the test sample, they compete by displacing the conjugates correlated with the amount of specific antibody present in the sample. The test has a number of advantages over indirect ELISA, as it is a multi-species test requiring fewer steps and without needing of anti-species conjugates: it is capable of differentiating between vaccinated and field infected animals (Schelling *et al*., 2003).

Polymerase Chain Reaction (PCR)

Non-culture detection methods, including fluorescent antibody test and immunoperoxidase staining, in addition to non-isotopic DNA hybridisation, offer rapid accurate detection in the absence of good cultures (Hopper *et al.,* 1989). Current PCR-based procedures provide a reliable aid for the detection of pathogens in tissues (Fekete *et al.,* 1992). However, for the accurate diagnosis extreme caution should be practiced, as none of the above-mentioned tests is suitable in all circumstances. For more information about PCR, see the Bovine Viral Diarrhoea Virus section below.

Prevention and control

The successful elimination of brucellosis in some countries is a consequence of well-conducted comprehensive control and eradication programmes, with screening and confirmatory tests and prompt removal of reactors, in addition to bio security and vaccination (Reviewed by Songer & Post, 2005).

Vaccination enhances the resistance of the animals in infected herds, and reduced doses of *B. abortus* strain 19 live vaccine is given to adults with systematic administration to heifers 4-12 months old. Other vaccines based on strains RB51 or 45/20 provides excellent immunity and recommended for the control of brucellosis in cattle (Chukwu, 1987; Stevens *et al.,* 1995), and the Rev-1 live vaccine secures lifelong immunity against *B. melitensis* in small ruminants (Blasco, 1997).

The control of brucellosis in camels in the UAE is hampered by lack of reliable demographic information, uncontrolled movement of camels across the borders to the Gulf States and the import of animals from other countries for racing. In this context, the camel population in Al Ain region has been estimated at around a hundred thousand heads (Moustafa *et al*., 1998). An increased understanding of the pathogenesis, host immunity interactions and genetic bases of persistence is the pivot of current research. Accordingly, choices of sub-unit vaccines or DNA immunization have shown efficacy and hold promise for a generation of highly effective genetically engineered vaccines (Kurar & Splitter, 1997). The optimised status of control for eradication is a persistent scientific challenge that should receive increased international financial and political support.

2. Bovine Viral Diarrhoea Virus (BVDV)

Epidemiology and pathogenesis

Bovine Viral Diarrhoea Virus (BVDV) is the prototypic representative of the genus *pestivirus*, family *Flaviviridae*, that includes classic swine fever and sheep border disease viruses. BVDV is a ubiquitous primary pathogen of cattle and 50-90% of cattle in the world have been exposed. The virion is an enveloped 40-50 nm particle, with a single stranded positive-sense, non-polyadenylated RNA genome, and is approximately 12.3 -12.5 kb in length. The genome has one large open reading frame (ORF) located between the 5 and 3 non-coding translated regions, encoding a single 4000 amino acid hypothetical polyprotein. The biogenesis of the ultimate structural and non-structural viral proteins occurs through proteolytic processing of the precursor polyprotein utilizing various cellular- and virus-originated peptidases (Collett *et al.,* 1991; Houe *et al.*, 1995; Paton *et al.,* 1995).

According to the detectable differences in the genome, two BVDV genotypes (I and II) have been described based on differences in the 5' non-coding region (5'NCR) nucleotides sequence (Pellerin *et al.,* 1994). The variations in the 5'NCR, the non-structural protein, and envelope glycoprotein E2 regions further characterised BVDV into sub-genotypes and updating of the pathogen phylogram (Collett, 1996). Moreover, the differentiation of BVDV genotypes, sub-genotypes and other pestiviruses is performed serologically either by differential virus neutralization test, or with monoclonal antibodies against E2, as well as genomic typing (Wensvoort *et al.,,* 1989; Ridpath, *et al.,* 1994a; Letellier *et al.,* 1999; Fulton *et al.,* 2003). BVDV-1 has at least five subspecies (1a-1e), predominantly originating from cattle, and occurs worldwide, whereas, BVDV-2 appears to be mainly restricted to North America, where it has been implicated in outbreaks of severe thrombocytopenic and hemorrhagic form of the disease (Rebhun *et al.,* 1989; Van Rijn *et al.,* 1997; Brownlie *et al.,* 2000).

Two distinctly differentiated BVDV biotypes, a cytopathic strain (cp), and a non-cytopathic strain (ncp) were characterized in culture cells (Meyers & Theil 1996). The ncp strain is regarded as the standard BVDV and spreads more extensively throughout host tissues than the homologous cp strains do and includes both virus 1 and 2 genotypes. Only the ncp biotype can trespass the placental barrier in pregnant seronegative females and establish a persistent infection (PI) in foetuses. If PI offsprings are superinfected by exogenous, genetically homologous cp strains, they may develop the fatal Mucosal Disease (MD); whereas, synergistic heterologous strains generate a chronic atypical form of the disease (Westenbrink *et al.,* 1989; Gronstol *et al.,* 1988). MD is a dramatic indication of the presence of BVDV in a herd and either of the two disease forms, the sudden or the late onset, results in poor survivability due to severe and profuse diarrhoea and progressive debilitation (Brownlie & Clark, 1993; Moening & Liess, 1995). Cp strains are postulated to generate a *de novo* form by mutation of ncp counterparts in PI animals (Moening & Plagemann, 1992; Meyers & Thiel, 1996).

The BVDV associated infections comprise five forms with variable degrees of severity, presented clinically as: diarrhoea, respiratory signs, thrombocytopenia,

and immunosuppression (Grooms *et al.,* 2002). However, unapparent infections are usual and an acute infection is generally subclinical and benign in most immuno-competent sero-negative animals. Profuse diarrhoea, oro-mucasal lesions, oculo-nasal discharges, agalactia and reproductive failure are reported from severe acute disease. In addition, clinical or pathological diffuse gastrointestinal ulceration, lymphoid necrosis, lameness and various congenital malformations, have been described. The hemorrhagic syndrome with thrombocytopenia may present as bloody diarrhoea, epistaxis, and oral and conjunctival haemorrhages (Corapi *et al.,* 1990; Liebler-Tenorio *et al.,* 2002). The association of BVDV with calf respiratory disease is a consequence to the immunosuppressive activity of the virus that potentiates the host's susceptibility to opportunistic co-infecting respiratory and enteric pathogens. Accordingly, pneumonia, enteritis and an increased incidence of udder infections are occasionally reported (Ri**c**her *et al.,* 1988; Lindberg & Emanuelson, 1997).

The foetopathogenic outcomes of diaplacental infections are determined by the pregnancy length at onset (For review see Sager & Ridpath, 2005). Early incidence in the first trimester results in embryonic death; whereas, incidence at mid-gestation, and before competent immunity develops, results in abortion or pre-natal dysmorphogensis. The teratogenic effect of the virus retards ocular and nervous tissue development, resulting in several congenital anomalies, collectively known as oculo-cerebellar syndrome (Bielefeldt-Ohmann, 1984). Foetuses infected during early pregnancy are borne immunotolerant, persistently viraemic and are candidates for developing fatal MD (Moening & Liess, 1995).

The main transmitters of BVDV are PI animals and they have an enormous impact on the epidemiology of the disease, as susceptible herd-mates contract the pathogen when exposed to their contaminated shedding (Duffel & Harkness, 1985). Iatrogenic transmission through contaminated needles, vaccines, semen, transferred embryos, and negligence of precautionary hygiene measures and practices, enhance the probability of virus introduction (Gunn, 1993; Barkema *et al.,* 2001).

The prevalence of BVDV in NWC and OWC has been described in several scientific works and the virus has been isolated from infected camels (see Table 2. BVDV is considered as the cause of death in both NWC and OWC and has been associated with cases of diarrhoea, stillbirth, intrauterine death, neonatal mortalities, and abortion. The virus also causes unthriftiness, weight loss and MD-like symptoms: some studies estimate the loss of newborn camel calves due to BVDV infections to reach 50% (Hegazy & Fahmy, 1997). Furthermore, congenital deformities, neonatal respiratory distress, and acute hemorrhagic gastroenteritis have also been reported in camelids (Mattson, 1994; Hegazy *et al.,* 1998; Belknap *et al.,* 2000; Wentz *et al.,* 2003; Abd-Elhakim, 2004).

Diagnosis

Diagnosis based on clinical features is difficult and unreliable owing to the marked clinical variations of BVDV infections (Baker, 1995). Therefore, laboratory diagnosis of BVDV is necessary to confirm the presence of the virus, and is successfully accomplished by direct detection of virus or viral components,

or serology. For an accurate interpretation of the results, clinical samples must be accompanied by herd history and vaccination status (Reviewed by Nettleton & Entrican, 1995).

Virus isolation (VI) and antigen detection

For viral isolation of cp strains, primary bovine cells are used. Increased sensitivity is best achieved by proper volume of *inoculum* and incubation period, and quality assured pestivirus free and species-specific susceptible cells: viraemia encountered in a few samples collected three weeks apart indicates PI (Brownlie, *et al.*,2000). Non-cytophathogenic strains are identified by immunofluorescence (IF), immunoperoxidase staining (IPX) and antigen-captured ELISA. Both , the cytopathogenic and the non-cytopathogenic biotype, have been cultivated from spleen and blood of MD affected animals and viral antigens have been detected by IF or IHC in biopsies or section of frozen tissues (Dubovi, 1990; Sandvik, 1999).

Polymerase Chain Reaction (PCR)

Reverse transcriptase (RT)-PCR, the current standard method for the detection of viral RNA, is more sensitive and efficient than dot blot hybridisation and virus isolation (Brock, 1991), which is affected by absence of live virus or presence of higher levels of antibodies in the material submitted (Zimmer *et al.*, 2004). The 5-NCR of the genome is the target for the selection of primers (Ridpath, 1993). The nested PCR is 100-fold more sensitive than the single step assay and detects the smallest concentrations of virus RNA in sera (Gruber *et al.*, 1994; Elvander *et al.*, 1998). In dairy farming, the test is used to detect PI animals and is performed on bulk tank milk, but is not valid for non- lactating animals (Graham *et al.*, 2001; Sandvik, 1999). The assay is validated by co-amplification of standard mimics, minimizing manipulation of samples with real time PCR, or single tube fluorogenic assays to enhance sensitivity and elimination of false results (Ballagi-Pordany & Belak, 1996; Bhudevi & Weinstock 2001).

Virus Neutralization (VN) test

The virus neutralization test has been considered as the “gold standard” test for the detection of antibodies against BVDV. However, it is expensive, laborious and impractical for large scale monitoring. The use of cp strains permits accurate identification of neutralizing antibodies, whereas immunoperoxidase staining (IPX) of infected cells is used for detection of virus infectivity inhibition of ncp strains (Fulton *et al.*, 1997). However, the absence of neutralizing antibodies in PI animals and interference with colostral immunoglobulins limit the diagnostic performance. Due to the antigenic heterogeneity of BVDV, the selected strain should be of reliable capacity for detecting the majority of infected animals within a particular area. The biotype can be accurately identified because of the difference between titres in infected animals and titres induced by vaccination (Jones *et al.*, 2001).

The Enzyme Link Immunosorbent Assay (ELISA)

ELISA (indirect and blocking) for either milk or serum is a rapid alternative to the VN test. The blocking ELISA is based on the competition between antibodies and a peroxidase coupled anti-p80 monoclonal antibody. The percentage of inhibition for each sample is calculated relative to negative controls. As the test has 97% sensitivity and specificity compared to VN tests (Beaudeau *et al.,* 2001), the serological status of dairy herds can be reliably determined with indirect ELISA on bulk milk. The antigen-captured ELISA, using monoclonal antibodies against the conserved p125/p 80, is a highly specific method, and correlates well with conventional VI methods, however, the presence of colostral antibodies may interfere with the identification of PI animals by both methods (Fenton, *et al.,* 1991; Shanon *et al.,* 1992; Katz & Hanson, 1987). From serological surveys in some tropical countries, antibodies to BVDV in dromedary camels give different prevalence rates, indicating the importance of the disease in this species (Table 2).

Control and prevention

The detection and elimination of PI animals and stringent biosecurity for preventing the introduction of the virus are key elements for designing efficient large-scale controls with or without vaccination (Bitsch & Ronsholt, 1995; Graham *et al.,* 2001). Testing bulk milk or a small number of animals, aged 6-18 months, will enable accurate prediction of PI animals in whole-herd screening protocols (Houe, *et al.,* 1995). *In utero* detection of PI foetus in newly introduced pregnant heifers reduces the establishment of PI family lines by blocking an important intra-herd vertical route of spreading (Lindberg *et al.,* 2001). After the removal of PI animals, maintenance of BVDV free herds is essential, as is mandatory testing before the introduction of new animals. Vaccination is an important preventive measure. The advantages and disadvantages of live and killed vaccines have been reviewed (van Oirschot *et al.,*1999). An alternative to vaccination is natural exposure, PI animals are used as bio-immunisers to protect young animals, which simultaneously confers protection to the older co-resident animals; however, risky repercussions cannot be ruled out (Pasman *et al.,* 1994).

The absence of BVDV antibodies in bulk milk or the absence of sero-conversion in spot-tests are considered sufficient criteria to certify a herd as BVDV free, and *vice versa*, and is a practice employed in eradication programmes (without vaccination) implemented in Scandinavian countries. Repeated multiple testing of BVDV-free herds, and methods that support self clearances in infected herds are essential complementary practices for the control of the disease (Bitsch & Ronsholt 1995; Lindberg & Alenius, 1999). Despite decades of vaccination with different types of vaccines, the optimal control and elimination of the disease is far from complete in many endemic regions, which is due to the diverse antigenicity of the virus, and complex pathogenesis.

3. Bovine Herpes virus-1 (BHV-1)

Epidemiology and pathogenesis

Bovine Herpes Virus type 1 (BHV-1) belongs to the *Herpesviridae* family, subfamily *alpha herpesvirinae*, genus *varicella* virus. BHV-1 is the aetiology of a number of clinical diseases, including infectious bovine rhinotracheitis, (IBR), infectious pustular vulvovaginitis (IPV) and balanoposthitis (IBP), as well as a systemic generalized form accompanied by encephalitis (For review see Straub, 2001).

The presence of BHV-1 subtypes may explain the dissimilar pathological and epidemiological features of the disease. Through genomic analysis, Metzler *et al.*, 1985, described three distinct subtypes: 1, 2a, and 2b. However, Rozimann *et al.*, 1992, reported a classification reflecting the subtypes based on tissue tropism: BHV-1.1 as a respiratory subtype; BHV.1.2 as a genital subtype, which were further divided by Miller *et al.*, 1995 into **a** and **b** groups; and BHV-1.3 (renamed BHV-5) as a neurovirulent encephalitic subtype. Cattle are the principal hosts; however, the virus has been reported in swine, goats, camels and wild ruminants, which are considered as reservoirs (Rimstad, *et al.*, 1992).

The epidemiological data and sero-surveys in various countries indicate a worldwide distribution. Prevalence rate in developed countries fluctuates depending on immunization practices and exposure of naïve herds to the infectious virus (Durham *et al.*, 1991). The latency of the virus in the sciatic, trigeminal ganglia, or tonsils following primary infections, with corresponding recrudescence and continuous or intermittent release, explains the virus-complicated epidemiology. Stress or administration of corticosteroids reactivates latent infections (Thiry *et al.*, 1987; Winkler *et al.*, 2000). Virus transmission is through contact, or indirectly through contaminated feed and drinking water. Therefore, cattle in feedlots have higher morbidity (20%-30% up to 100%), as husbandry systems potentiate virus emergence, transmission and maintenance, than the 3-8% in dairy heifers affected by IPV (Radostits & Arundel, 2000). In uncomplicated respiratory form, the average mortality is 10% and in the reproductive tract form, it is negligible. BHV-1 related-diseases complex accounts for huge economic losses due to reduced milk production and dropped feed efficiency, IPV/IBP-induced infertility, abortion, neonatal mortality, and reduced conception (Kaashoek, 1996).

BHV-1 causes both clinical and sub-clinical infections. The clinical disease has two major pictures: a respiratory form of varying severity affecting animals 6-months to 2-years-old, and a venereal form affecting adult males and females genital organs (van Oirschot *et al.*, 1993; Hage *et al.*, 1996).

The respiratory (IBR) form

This form is typical rhinotracheitis and is usually acquired through aerosols or droplets. It involves the entire respiratory tract with increased severity in younger calves. Fever and depression progress to rhinitis, laryngitis, and tracheitis with dyspnoea, deep coughing, and hyperexcitability. Moreover, nasal mucosa is extensively hyperaemic and ulcerated, with necrotic foci in the nostrils and

muzzle. Profuse mucopurulent bloodstained discharges are usually seen. Occasionally, conjunctivitis with corneal involvement and abundant lacrimation may be the sole clinical abnormality. Abortion or stillbirth of extremely autolized foetus during the last trimester is common in non-immunized pregnant cows. Infected animals exhibit elevated susceptibility to fatal broncho-pneumonia, or endometritis in the genital form, subsequent to secondary bacterial infections (Babiuk *et al.,* 1995). IBR in newborn calves is a fatal generalized systemic disease, characterized by gastroenteritis with diarrhoea, and oculitis. Virus neurotropism leads to meningo-encephalitis with in-coordination and convulsions, which represents another variety of BHV-1 infection.

The genital (IPV) form

Natural service or infected semen in artificial insemination centres and contaminated milking machines are implicated in the IPV transmission most commonly observed in diary cows. Swollen and exudating *labia vulvae*, necrotic ulcers and formation of pustules in the vestibular mucosa, with painful and frequent urination, is described. In males, continuous discharge, and persistent lesions in the penis are observed in bulls affected by IBR. There is rare evidence of simultaneous onset of both forms in an individual animal, or a particular herd, but generally, one type is predominant (Murphy *et al.*, 1999).

The pathogenesis is mostly encountered at the mucus membranes of the upper respiratory tract or of the vagina and prepuce. Several envelope glycoprotein and cellular receptors mediate a viral multistep entry process into the cells (Mettenlieter, 1994). Rapid initial replication occurs and produces local cytolysis; the resultant cell destruction is responsible for the acute symptoms and the leukocyte associated primary viraemia that lead to virus dissemination to different host tissues (Engels & Ackermann, 1996).

Evidence of BHV-1 infection in OWC is scarce but a higher prevalence has been reported in NWC, particularly in llamas and alpacas, and in association with bronchopneumonia, encephalitis, and progressive cough. A higher prevalence of the disease has been reported when grazing pastures are shared with other ruminants (Wernery & Kaaden, 1995; Williams *et al.*, 1991; Mattson, 1994; Rivera *et al.*, 1987; Picton, 1993).

Diagnosis

Virus isolation

The clinically observed signs of the reproductive form and conjunctival lesions provide a relatively reliable basis for tentative diagnosis. However, several laboratory methods, including electron microscopy, virus isolation, immunofluorescens (IF), immunoperoxidase (IPX) staining, and antigen ELISA, are essential for the identification and confirmation of the different forms of the disease. In the early acute febrile stages, the virus is isolated from samples of nasal, ocular, and genital swabs. The cytophathic effect (CPE), in cultures of various cell types of bovine or swine origin, indicates the presence of the virus. In addition, *in situ* demonstration of viral antigens by IF or IPX assays is usually

practiced and is applicable for post-mortem collected tissues (Kaashoek *et al.*, 1994).

Virus Neutralization (VN) test

The Virus Neutralization (VN) test is the utmost confirmative test and is applicable for bovine kidney, lung, tracheal, and other cell lines of bovine origin. The sensitivity of the test can be maximized by a prolonged virus/serum incubation period (Bitsch, 1978).

ELISA

Different ELISA are used for antibody detection in individual or pooled blood sera, as well as in bulk milk, and is useful for determining herd status. The optimal accuracy of the tests means they are a reliable alternative to the VN test in areas of high prevalence (Pritchard, 2001). Recent infections can be successfully diagnosed by an IgM-ELISA, where immunoglobulin IgM predominate the primary immune response. Furthermore, indirect ELISA has 100% sensitivity when undiluted test serum samples are used. Therefore, the test can detect low levels of specific antibodies undetected by other conventional assays (Lemaire *et al.*, 1995). The commercially available blocking ELISA kits with monoclonal antibodies can discriminate immunized from naturally infected cattle in regions where marker vaccines are used. (Perrin *et a1.*, 1993; van Oirschot *et al.*, 1997).

PCR

PCR is used for viral DNA detection *in semen* and is a valuable complementary technique for classic viral detection methods (Vilcek *et al.*, 1994; van Engelenburge *et al.*, 1995).

Control and prevention

Unpredictable virus occurrence, latency and sub-clinical shedding are major obstacles jeopardizing any control programme, although occasionally, natural exposure to the virus confers solid immunity. Eradication schemes without vaccination have been successfully implemented in Scandinavian countries and Switzerland (for review see Radostits and Arundel, 2000). The strategy adopted was based on prompt notification and prohibition of vaccination to unmask natural or latent infections, in combination with annual testing and trade restrictions (Ackermann *et al.*, 1990; De Wit *et al.*, 1998). The feasibility of such programmes is doubtful in countries with high prevalence rate, where restriction on animal movement and culling of infected animals face practical difficulties due to the extensive nature of animal production. Therefore, in many countries vaccination has been used (Paton et al., 1998). The currently used vaccines include conventional inactivated or modified life vaccines, in addition to subunits and marker vaccines, which allow discrimination of animals infected with wild-type pathogens from the vaccinates. Although all have proved beneficial in certain conditions, several vaccination disadvantages are still reported (Nettleton et al., 1984).

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Introduction to the research report

Camels are essential sources of human food and constitute important supplies of milk, meat and wool, in addition to their use in the traditional popular racing sport, which is important to the rapidly growing tourism industry in Arab countries. In recent decades, the numbers of Camels has steadily increased due to the introduction of modern husbandry methods and efficient veterinary services. The scarcity of scientific publications on infectious diseases in camels has urged scientists to focus more attention on research and expand knowledge of this species. Camels are frequently infected with *Brucella abortus* because of their contact with other ruminants and consumption of unpasteurised bovine milk. In addition, the local habit to drinking raw camel's milk is of major public health concern as camels have varying degrees of susceptibility to BVDV and Bovine Herpes Virus type 1 (BHV-1), and their role in the epidemiology of these diseases requires more attention.

This work attempted to study the occurrence of the three important pathogens in serum samples from camels in the Al-Ain region, United Arab Emirates. Although, both viral pathogens do not directly threaten human health, together with *B. abortus* they are serious immunosuppressive pathogens and cause economic impact to animal production and international trade.

Aims of the study

The general aim of this work was to use ELISA for determining the presence of antibodies against *B. abortus*, BVDV, and BHV-1, which were suspected of affecting the reproductive system of camels in the Al-Ain region of the United Arab Emirates.

The specific aims of the research reported in this thesis, were:

- To introduce ELISA for the detection of *B. abortus*, BVDV, and BHV-1 in camelids, and to identify their role in transmission and spread in the camel population.
- To assess the reliability of both competitive and blocking ELISAs as diagnostic tools for use in more comprehensive studies
- To study the occurrence of the three diseases in groups of animals with different management systems. This will assist the assessment of the efficacy of control and eradication programmes already initiated by the local authorities and provide recommendations for strengthening future control of camel diseases in the region

Research report

A survey of antibodies to *Brucella abortus*, Bovine Viral Diarrhoea Virus (BVDV) and Bovine Herpes Virus-1 (BHV-1) in camels from the Al-Ain region in the United Arab Emirates

Abstract

Serum samples from 812 camels of both sexes and different ages were randomly collected from the local abattoir and livestock market and from10 private holdings in the Al Ain region of the United Arab Emirates. A commercial competitive ELISA, based on a monoclonal antibody, determined the presence of *Brucella abortus* antibodies, and a commercial blocking ELISA was used for the detection of antibodies to BVDV and BVH-1. Suspected or non-specific positive results were retested by confirmatory tests such as Complement Fixation or Virus Neutralization tests as required. The results indicated that no sera from the 812 camels were positive for any of the three pathogens. This highlighted the need for further comprehensive studies and a reconsideration of the sampling on a defined epidemiological basis, and with cut-off values for commercial ELISA kits originally designed for other species, and which are used for testing camel sera. A performance evaluation of commercial ELISA kits used on camel sera should be a priority for future epidemiological studies.

Introduction

Single humped camels, *Camelus dromedarius*, together with the two humped camels *Camelus bactrianus*, inhabit wide areas throughout the world, including the Arab Gulf States, and have an important impact on people's life as essential sources of human food and entertainment. Camels have a unique adaptation to harsh environmental conditions such as those existing in Arabian Gulf States. The adoption of modern husbandry and management methods and availability of efficient veterinary services has steadily increased the number of camels as an alternative source of milk and meat, in addition to the widely popular racing sport. The accurate determination of the incidence of brucellosis in the UAE, including the Al-Ain region, faces several problems in part due to the absence of reliable demographic information on camel population and the uncontrolled movement and import of camels from other countries. As the camel population is roughly estimated as a hundred thousand heads, the animal health authorities are forced to give more support for determining the health status of camels in the region (Moustafa *et al.*, 1998).

Brucella abortus (B. abortus)

Brucella abortus was first reported in camels in 1931 (Solonitsyn, 1949). Camels are considered as having relatively high susceptibility to *B. abortus* infection, especially when they are in contact with infected large or small ruminants (Hashim *et al*., 1987; Radwan *et al.,* 1995). The incidence and prevalence of the disease has been clearly related to the type of breeding and husbandry practices (Richard, 1980; Abbas & Agab 2002). A low prevalence occurs in nomadic or extensively kept pastoralist camels, whereas, a higher prevalence is found in intensively or semi-intensively reared animals. The duration of inter-calving intervals, which is longer in nomadic camels, appears an important factor. Similar findings are reported from Saudi Arabia and Sudan indicating the same difference in incidence pattern (Abu Damir *et al*., 1984; Wernery & Wernery 1990; Radwan *et al*., 1992; FAO, 2003).

Different biotypes of *B. abortus* and *B. melitensis* are involved in camel brucellosis (Higgins, 1986; FAO 1986; Radwan *et al.,* 1991; Wernery & Kaaden, 1995) and different prevalence rates are reported in several countries (FAO 1986). The prevalence of brucellosis in camels in various countries is presented in Table 1. Abortion is the main clinical finding, but placental retention has not been described (Radwan *et al*., 1995; Fowler, 1998). Pathological changes are associated with the disease, including lesions in ovario-bursal, hydrobursitis and granulomatous endometritis and endometrial fibrosis (Agab, 1993; Nada & Ahmed, 1993).

Most data on *B. abortus* infection in camels are based on serological surveys; although, isolation from blood, milk, lymphoid tissues, and diagnosis through immunohistochemical methods has been described (Abu Damir *et al*., 1989; Gameel *et al*., 1993). Besides the conventional tests, such as slide agglutination (SAT), buffered-plate agglutination (BPAT), buffered-acidified plate antigen (BAPAT) and card or Rose Bengal tests (RBT), the Complement Fixation Test (CFT) is considered as the most sensitive diagnostic test for brucellosis in camels. Moreover, it has often been considered as a confirmatory test for brucellosis, with four times higher sensitivity than the agglutination test (Shumilov, 1974; Nielsen, *et al.,* 1984; Alton, *et al.,* 1988; Wright & Nielsen, 1990). Indirect and competitive ELISA have successfully detected antibodies to *B. abortus* in milk and sera (Starten *et al*, 1997).

The C-ELISA used in the present study, is a multi-species assay for the detection of antibodies to *B. abortus* or *B. melitensis* in serum. This test naturally discriminates infected animals from those vaccinated with strain 19 and animals infected with serologically cross-reacting bacteria such as *Yersinia enterocolitica* and different serotypes of *Salmonella* and *E.coli.* The performance of C-ELISA is comparable to the CFT test, therefore, it can reasonably be used as a sole test for the diagnosis of brucellosis (Nielsen *et al.,* 1995). As data on the prevalence of camel brucellosis in most countries is limited and not updated information, there are many gaps in the knowledge of the epidemiology, pathogenesis and clinical

patterns of the disease, which therefore merit further investigation (Radwan *et al.*, 1992).

Bovine Virus Diarrhoea Virus (BVDV)

BVDV is an important pathogen causing significant economic losses primarily due to reproductive failure, infertility and neonatal mortality. The virion is an enveloped, 40-50 nm virus member of the family *Flaviviridae*, genus *pestivirus.* The genome is a single-stranded positive-sense RNA molecule, approximately 12.5 kb in size. The virus has two genotypes, BVDV-1, with a worldwide distribution, and BVDV-2 that appears geographically restricted to the U.S. and Canada. The virus has two biotypes: cytopathogenic "cp", and non-cytopathogenic "ncp" types, which are differentiated according to the presence or absence of microscopic pathological changes in cell cultures (Reviewed by: Ridpath, 2005).

Tthe involvement of type -1 in severe outbreaks of the disease has been reported. In addition, the virus is an immunosuppressive agent with an affinity for the lympho-reticular system, which enhances the host susceptibility to other pathogens i.e. Herpesviruses (Potgieter *et al*., 1984; Bolin *et al*., 1985; Peterhans *et al*., 2003). The prevalence of BVDV infection in OWC and NWC has been described and the virus has been isolated from infected camels (Evermann *et al.*, 1993; Mattson, 1994; Hegazy *et al*., 1998).

In some tropical countries, antibodies against BVDV in camels reveals different rates of prevalence, as highlighted in Table 2, which indicates the importance of the disease in camelids. The cp biotype has been isolated from lymphoid tissues, spleen, brain and kidney in bovine kidney cells, and detected by immuno-histochemical methods in different organs (Wernery & Kaaden 1995; Belknap *et al.*, 2000).

BVDV is considered as the cause of death in both OWC and NWC and is associated with cases of diarrhoea, stillbirth, intrauterine death, neonatal mortalities, and abortion. The virus also causes unthriftness, weight loss and MD-like symptoms and is responsible for approximately 50% loss of newborn camels in Egypt (Hegazy *et al*., 1995). Furthermore, congenital deformities and poor viability, neonatal respiratory distress, acute haemorrhagic gastroenteritis is also reported (Belknap *et al.*, 2000; Hegazy & Fahmy 1997; Hegazy *et al*., 1998).

Bovine Herpes Virus-1 (BHV-1)

Bovine Herpes Virus, BHV-1, is the causative agent of infectious bovine rhino tracheitis (IBR), abortion, and, in rare cases, encephalitis in cattle and IPV/IBP in bulls. The virus comprises respiratory, genital, and encephalitic subtypes known as BHV-1.1, 1.2 and 1.3, which has been renamed BHV-5 (Roziman *et al*., 1992). IBR acts also as an immunosuppressive agent predisposing the host to a several secondary bacterial invasions (Reviewed by Straub, 2001) and is most often characterised by respiratory syndromes manifested by coughing, rhinitis, fever, and broncho-pulmonary complications, and, has a propensity to remain latent after the primary infection, and might be later provoked after a viral reactivating event. The high losses due to this viral disease justify the meticulous monitoring of susceptible herds for exposure to the virus.

Evidence of BHV-1 occurrence in OWC is scarce. However, Schwyzer & Ackermann, 1996 and Bildfell *et al.*, 1996, describe encephalitis and blindness in camels caused by Equine herpesvirus-1 EHV-1, which has a close genetic relation to BHV-1. Higher incidences of BHV-1 associated diseases are reported in NWC than in OWC counter parts: bronchopneumonia, encephalitis, and progressive cough are evident in llamas and alpacas (Rivera, 1987; Rebhunn *et a*l., 1988; Williams *et al*., 1991; Picton, 1993; Mattson, 1994). Higher prevalence has been recorded when grazing pastures are shared with other ruminants. The lack of positive results to BHV-1 in experimentally infected OWC is perhaps attributed to the difficulties in detecting natural or experimentally–induced humoral responses to BHV-1 in some laboratories. However, a prevalence of 13.5% is reported in samples of 171 dromedaries in Egypt (Moussa *et al.*, 1990) and antibody titres (1:5) are reported in 5.8% of dromedary samples screened in Tunisia (Wernery & Kaaden, 1995).

Materials and methods

Animals and serum samples

The study comprised 812 camels of nomadic and intensively reared animals, which represented different husbandry and management systems. The sampling frame included 392 males and 14 females from the central abattoir, 108 males and 102 females from the camel markets and 111 males and 85 females from 10 privately owned farms. The age of the animals ranged from 1-12 years in both sexes. The geographical origins, and sex, were recorded. All animals were clinically normal at the time of sampling, May-July 2002. Table 3 summarizes the origin, sex, and total numbers of sampled animals.

Sampling procedures

Plain vacutainer tubes without anticoagulant were used to take 5-7 ml of blood from the jugular vein. Serum was separated from the coagulated blood by centrifugation at 1000x g for 10 minutes. The sera were inactivated by heating in a water bath at 56ºC for 2-3 hours and stored at -20 ºC until use.

Serological tests

Competitive ELISA for detection of antibodies against *B. abortus*

A commercial *Brucella abortus* C-ELISA supplied by SVANOVA Biotech, Uppsala–Sweden was used. The kit procedure was based on a solid phase competitive ELISA, where samples were exposed to *Brucella abortus* smooth lipopolysaccaride (S-LPS) coated wells on microtitre plates, together with a mouse monoclonal antibody (mAB) specific for an epitope on the o-polysaccharide portion of the S-LPS antigen. After incubation, the microplate was washed and goat anti-mouse IgG antibody conjugated with horseradish peroxidase was added. The latter binds to any mABs bound to the S-LPS on the plate. Colour

development is due to the conversion of the substrate by the conjugate. The mean optical density (OD) values at 450 nm of the controls and the tested samples were used to calculate the percent inhibition PI with the formula: PI=100- mean OD samples or control times 100/ mean OD of conjugate controls CC. The remaining steps of the test followed the manufacturer's instructions (SVANOVA *Brucella* - Ab C-ELISA manual), including the interpretation of the results.

Blocking ELISA for detection of antibodies to BVDV- BHV-1 (IBR)

All sera were examined for the presence of BVDV and BHV-1 (IBR) antibodies with commercial blocking-ELISA (B-ELISA) kits manufactured by Institute Pourquier, France (http://www.institut-pourquier.fr.). The principle of the test was based on the P80 protein of the virus coated on wells of a microplate by means of a specific monoclonal antibody "WB103". If antibodies against BVDV were present in the sample, they formed a bovine (or camel) antibody-P80 complex, through which the P80 became "masked". After washing, a monoclonal antibody "WB112" (directed to another epitope of P80) coupled to peroxidase was incubated in the wells. In the presence of antibodies in the sample, the P80 protein sites were "masked" and the conjugate could not bind to the corresponding epitope, but the conjugate could bind to the P80. After further washing, the substrate was added to the conjugate, forming a blue compound, which became yellow after the blocking step if the sample was negative.

The general principle of the B-ELISA for the detection of antibodies against BHV-1 is exactly similar to the procedure followed for the detection of antibodies against BVDV. Here, the wells of microplates were precoated with an ultra purified BHV-1 lysate. Any antibody specific to the glycoprotein gB of the virus present in the sample formed a gB-antibody immune complex, which effectively masked the gB sites.

In order to consider the BVDV ELISA test as valid, the OD of the negative control (NC) is at least 0.800 and the PI of the positive control (PC) <20%.For IBR ELISA a minimum OD of 0.800 for NC and PI<30% is required for a valid test. PI for both tests was calculated by the equation: OD450 nm of analysed serum/mean OD 450 nm of the NC x 100.

The details of both tests followed the manufacturer's instructions and the validation criteria and interpretation of results is summarised in Table 4.

Results

Brucella abortus

Sixty-six out of 812 samples revealed a percent inhibition ranging from 20-25. The same results were found in two repetitive tests. The distribution of the samples with the above mentioned percent inhibition was 18 samples from the abattoir, 16 from the market and 32 from the private farms. As range of the percent inhibition values (20-25) was not clear-cut (Table 4), it might be an indication of the presence of low levels of specific antibodies. The 66 samples were re-tested for confirmation with Complement Fixation Test: none of the samples was f positive with CFT. The test was performed following the protocols of the Department of Bacteriology Swedish National Veterinary Institute (SVA) Uppsala-Sweden.

BVDV, BHV-1 tests

Three out of 210 samples obtained from the camel market revealed OD values of 0.220, 0.645 and 1.046 with corresponding calculated percent inhibition values of 13, 38 and 55. These samples were suspected as being positive for BVDV. A further two out of 210 samples, also from the camel market, gave OD values corresponding to 0.79 and 0.116 with corresponding percent inhibition values of 50 and 7. They were also considered suspected as being positive for BVDV, and were reanalysed for confirmation with the VN test at SVA laboratories: all results were confirmed as negative.

Discussion

This study was designed to determine the occurrence of antibodies to *Brucella abortus*, BVDV and BHV-1 in camels from the Al-Ain region in the UAE. As camels are reared together with ruminants (cattle, sheep and goats), collecting data on the prevalence of these diseases is important not only for the economic impact of the diseases but also because the camels are a source of food, and in the case of brucellosis, because of the zoonotic nature of the disease.

After confirmation of suspected results in some samples, no antibodies to *B. abortus*, BVDV or BHV-1 were detected in the 812 serum samples, despite the serum samples being collected from animals of different ages, sex, and husbandry systems.

Previous studies on camel brucellosis in the Al-Ain region have shown a declining prevalence of the disease. During the period 1990-1996, Moustafa *et al* (1998) reported a gradual reduction of the prevalence of brucellosis from 5.8% in 1990 to 0.1% in 1996: this was considered due to the reduction in the prevalence of the disease in other ruminants. In a study by Afzal & Sakkir (1994), the decrease in prevalence of brucellosis was attributed to age in a majority of camels in the Al-Ain region, as they are no longer used for breeding; however, Fazil & Hofmann (1981) consider it the spontaneous recovery occasionally reported in non-breeding female camels.

The results in this study agreed with those published in the Annual Bulletin of Al-Ain Department of Agriculture and Animal Resources (2002), where no cases

of brucellosis were reported in the 1187 samples tested during the period 2001-2002. A few investigations have revealed a prevalence of 1.5% in camels in the Abu Dhabi area and 2% in free grazing dromedaries and 6% in racing dromedaries in the whole country. These findings were attributed to the fact that racing camels are fed with un-pasteurised dairy cattle milk (Afzal & Sakkir, 1994; Wernery & Wernery, 1990). Although the assessment of the prevalence based on age and sex, is beyond the scope of this survey, the findings agreed with the results obtained by Radwan *et al* (1992), who report no difference in the prevalence of brucellosis among male and female camels**.** However, the results did not reveal any differences in brucellosis occurrence among the sampled animals, and diverged with the findings of other authors who indicated an influence of husbandry systems upon prevalence that was rather high in the intensively reared camel production (Radostits *et al.,* 1994; Teshome *et al.,* 2003).

The C-ELISA used in this study was based on a monoclonal antibody against a single antigen epitope and therefore enhanced sensitivity. The partial and very low competition, reflected in 66 samples (percent inhibition 20-25), could be attributed to sub-optimal infectious doses of the pathogen, or due to varying stages of infection, where low avidity antibodies produced early during the primary immune response, were not able to compete with the monoclonal antibodies or with cross reacting ones. Despite the results obtained, the incidence of "Malta Fever" among individuals working with camels is common, and cases of sporadic abortion in camels raised in close contact with other ruminants are still reported in the area, although, the source of both human and camel infection was unclear. This could be by people acquiring the infection from other sources, and the cases of abortion in camels could be caused by other organisms or factors not covered by this study.

BVDV infections in cattle have an impact on the economy of the farmers; thus, is essential to include camels in the animal health monitoring programmes of the country, not only because cattle and sheep are the natural hosts for BVDV, but also because other animal species are in contact with them or are raised in the same vicinity. ELISA has excellent specificity and sensitivity for detecting of BVDV antibodies and a close agreement with VN in bovine sera as reported by Abd-Elhakim (2004). The convenient technical features, adaptability for automation, and rapid results, are other advantages that favour the application of ELISA for screening large numbers of samples, compared to the expensive culture systems, and time consuming work needed in VN (Evermann, *et al.,* 1993; Chu *et al.,* 1985; Howard *et al.,* 1985).

In two surveys on dromedaries in Sudan (see Table 2), the prevalence of BVDV was found to be 15.5% in 1987 and 15.7% in 1989 (Bornstein & Musa 1987; Bornstein *et al.,* 1989). In other studies, the prevalence of BVDV was 3.9% in Tunisia (Burgemeister *et al.,* 1975, 6.7% in Oman (Hedger *et al*., 1980), and 3.4% in Somalia (Bornstein, 1988). In the UAE, Wernery & Wernery (1990) reported a prevalence of 3.6%. In serum samples of 552 racing and breeding camels, a prevalence of 6.4% was recorded for breeding camels and only 0.5% for racing dromedaries: this prevalence pattern was attributed to the densely crowded

breeding herds closely located to cattle herds. Reports from Egypt present a range of rates: 11% (Hegazy *et al.,* 1993), 4.3% (Tantawi *et al.,* 1994) and 52.5%, which is an extremely high prevalence: no explanation on these results are given (Zaghana, 1998).

The detection of antibodies against BHV-1 in camelids difficulties problematic, even in experimentally induced infection, and serocoversion was not always observed (Wernery & Kaaden, 1995). The results presented here are in agreement with the findings of Paling *et al.* (1979), Agrimi, *et al.* (1982), Bornstein & Musa (1987), Bornstein (1988), and Wernery & Wernery (1990), where no specific antibodies against BHV-1 were detected. Contrary to the results observed in OWC, evidence of BHV-1 isolation and seroconversion in NWC has been reported by Rivera (1987), Picton (1993), Rosadio *et al.* (1993) and Mattson (1994). Moreover, Wernery & Kaaden (1995) reported a prevalence of 16.2% in Peruvian alpacas, which declined to 5.1% in subsequent years when a change in production systems was introduced and the animals were reared in separate pastures not shared with other ruminants. If these finding are to be generalized, the close contact between camel and cattle herds supports the inter-species transmission of viral infection.

Although C-ELISA and B-ELISA were applied for the first time for the screening of camel sera for antibodies against the three pathogens, the techniques displayed excellent agreement with the corresponding gold standard tests (CFT and VN). Thus, ELISA is reliable as a single technique for the detection of antibodies to B. abortus, BVDV and BHV-1 antibodies in camels; although, reliability would be enhanced if the technique were used in combination with conventional techniques such as IHC.

Finally, the current study was limited due to the difficulties faced during the sampling. The ultimate consequence of the results obtained cannot be easily predicted. For a better understanding of the importance of brucellosis, BVDV and BHV-1 in the whole country in general and in areas of low prevalence in particular, a wider study is recommended.

Table 1. Prevalence of brucellosis in camels in various countries (performed by Complement Fixation Test).

Country	**Prevalence%**	**References**
Egypt	23.1	Nada 1984
Sudan	8	Osman & Adlan 1987
Somalia	5.9	Bornstein *et al.,* 1988
Kenya	14.0	Waghela *et al.,* 1978
Mongolia	1-3.7	Shumilov 1974
Niger	8.3	Bornarel & Akakpo 1982
Libya	4.1	Gameel *et al.,* 1993
Iran	8	Zowghi & Ebadi 1988
Saudi Arabia	8	Radawan *et al.,* 1995
Kuwait	14.8	Al-Khalaf & El-Khaladi 1989
Oman	3.6	Harby & Ismaily 1995

Table 2. Prevalence of BVDV in camelids from the Old (OWC) and New World, (NWC).

Countries	**Camels**	**Prevalence %**	**References**
Sudan	OWC	15.5 15.7	Bornstein & Musa 1987 Bornstein *et al.,* 1989
Tunisia	OWC	3.9	Burgemeister *et al.,*
Oman	OWC	6.7	Hedger *et al.,* 1980
Somalia	OWC	3.4	Bornstein, 1988
UAE	OWC	9.2-3.6 6.4 - 0.5	Wernery & Wernery 1990 1998 DCVRL*
Egypt	OWC	11-23 4.3 52.5	Hegazy *et al.,* 1993 Tantawi *et al.,* 1994 Zaghhana 1998
USA	NWC	4.4	Mattson, 1994
Peru	NWC	11.11	Rivera *et al.,* 1987
Argentina	NWC	2.05	Puntel *et al.,* 1999

*Dubai Central Veterinary Research Laboratory.

Table 3. Origin, sex, and total numbers of sampled animals.

Place	**Males**	**Females**	**Total**
Abattoir	392	14	406
Camels market	108	102	210
Private farms	111	85	196
Total	611	192	812

Table 4. Validation criteria for C-ELISA (*B. abortus)* and B-ELISA (BVDV and BHV-1**).**

Pathogens	***B. abortus* (C-ELISA)**	**BVDV (B-ELISA)**	**BHV-1 (B-ELISA)**
Validation criteria for controls	OD for CCs 0.75-2.0 PI for PC 90-110	OD, NC >0.800 PC, PI <20%	OD, NC >0.800 PC, PI <30%
Inter-pretation of results	PI= 90 -110 positive PI= 35-65 weak positive PI= 10-15 negative	PI >50% negative PI 40%-50% doubtful PI <40% positive	PI >55% negative PI 50-55% doubtful PI <50% positive

OD= Optical density; PI= percent inhibition; CCs= controls; NC= negative control; PC= positive control.

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