

# SEROEPIDEMIOLOGICAL STUDIES FOR THE DETECTION OF ANTIBODIES AGAINST 8 INFECTIOUS DISEASES IN DAIRY DROMEDARIES OF THE UNITED ARAB EMIRATES USING MODERN LABORATORY TECHNIQUES-PART II

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## ABSTRACT

A total of 1119 dromedary sera (578 dams, 541 calves) were tested for 8 different infectious diseases using commercially available cELISAs or other tests. No antibodies were detected for Enzootic Bovine Leukosis. Antibodies were detected to Rift Valley Fever (0.35%), Bluetongue (21%), U.C. Equine Rhinitis A (5.7%), Camel pox (66%), Bovine Viral Diarrhoea (1.6%), Leptospirosis (12%) and Neosporosis (13.7%).

**Key words:** Dairy dromedaries, infectious diseases, serology

Infectious diseases can play an important role in the economic viability of dairy enterprises. This is especially true for the bovine dairy industry but may also gain importance for camel dairy farms. In many western countries, cattle health schemes for infectious diseases like Johne's disease, IBR, BVD and leptospirosis are licensed and regulated by 'Cattle Health Certification Standards' which is a self-regulatory body aiming to help control and eradicate non-statutory diseases (Winden and Pfeiffer, 2008).

Such schemes have led to the eradication of certain diseases, and for certified disease-free animals, a premium is paid when traded. However, before these animal health schemes can be applied to camel dairy farms, test methods which are generally used for cattle must first be evaluated for camels.

Over the last 2 years, we have evaluated serological tests for several infectious diseases which one day may become important for the camel dairy industry.

The first results of these investigations have been published in Part I (Wernery *et al*, 2008a). In the second part of our seroepidemiological studies we have concentrated our research on the prevalence and test evaluation of a further 8 infectious diseases for dromedaries, the results of which are reported here.

## Materials and Methods

A total of 1119 dromedary sera (578 dams, 541

calves) belonging to the Emirates Industry for Camel Milk and Products (EICMP) in Dubai were tested for 8 infectious diseases using different test methods. For leptospirosis only, 164 adult dromedary of the total 541 sera were examined.

## Viral Diseases

**Rift Valley Fever (RVF)-** The RVF Inhibition ELISA was purchased from BDSL, UK. It is a competitive ELISA for the detection of RVFV antibodies in humans, domestic and wild ruminants. This ELISA uses an anti-rabbit horseradish peroxidase (HRP) labelled conjugate which binds the IgG rabbit antibody. The conjugate is not directed against the animal species tested, and therefore, the test can be used for different animal species including camelids (Paweska *et al*, 2005).

For the production of positive control sera, 2 male 3 year-old dromedaries were administered 2 ml of an attenuated RVF vaccine purchased from Onderstepoort, South Africa. The vaccine was given subcutaneously twice within a time frame of 4 weeks.

Negative sera were obtained from 3 adult female dromedaries kept at CVRL, Dubai.

**Bluetongue (BT)-** The BT ELISA was purchased from IDVet, Lillidale Diagnostics, UK. It is a competitive ELISA which can be used for any animal species including camels. The ELISA is designed to detect

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antibodies directed against the Bluetongue virus vp7 protein. The conjugate is an anti-vp7-HRP conjugate.

Positive sera were produced by vaccination of 2 male 3 year-old castrated dromedaries kept at CVRL with an inactivated BT vaccine from Merial, France (BTVPURAI Sap2-4<sup>®</sup>). Both dromedaries were subcutaneously injected with 4 ml of vaccine each time within a 3 week period. Negative sera were obtained from 5 female 8 year-old dromedaries kept at CVRL, Dubai.

**Equine Rhinitis A virus (ERAV)-** Camel sera were tested by the serum neutralisation test (SNT) described by Wernery *et al* (2008b). Sera for antibodies against ERAV were tested in 96-well microtitre plates. A triplicate 2 fold dilution series of each serum, diluted from 1:2 to 1:128 in MEM medium without FCS was prepared on the plates. An equal volume of the reference ERAV strain NM-11/67, containing 100 TCID<sub>50</sub> was added to each of the serum wells. A virus control titration was included in each test to confirm the virus dose used. Serum/virus mixtures were incubated for 1h at 37°C. Vero cells at a concentration of 2.0 x 10<sup>6</sup> cells ml<sup>-1</sup> were added to all wells. Plates were then sealed and incubated for 3 days at 37°C and read microscopically on days 2 and 3. Titres were calculated as the reciprocal of the last dilution of serum in the serum/virus mixtures showing CPE at the 50% end point (Finney, 1964).

**Camelpox (CP)-** Dromedary sera were tested with the SNT using a similar approach as mentioned for ERA, and described by Pfeiffer *et al* (1998). The *Orthopoxvirus cameli* used for the SNT was isolated from a dromedary suffering from general camelpox. It was designated as strain 298-2-89 Ducapox vaccine strain. Vero cells were used for the SNT.

**Bovine Viral Diarrhoea (BVD) and Mucosal Disease (MD)-** The antibody ELISA was bought from Institut Pourquier, France. The BVD antibody ELISA is designed to detect specific antibodies in serum and milk samples. It uses a monoclonal antibody 'WB112' coupled to HRP. The monoclonal antibody is directed to the epitope of P80 of the BVD virus. Therefore, it can be used for any animal species.

Positive dromedary sera were produced by vaccination of 2 animals kept at CVRL with a BVD inactivated vaccine 'Mucobovin<sup>®</sup>' from Merial, France. Four ml of the vaccine were given subcutaneously twice within 3 weeks. Negative sera originated from 2 castrated male dromedaries, also kept at CVRL, with no vaccination history.

**Enzootic Bovine Leucosis (EBL)-** This ELISA kit was bought from Institut Pourquier, France. This

blocking ELISA detects specific antibodies directed against the BLVgp51 antigen. It uses a monoclonal anti-gp51 HRP conjugate. This conjugate is not directed against the species tested and can therefore, be used for camelids.

Since there is no vaccine available for EBL, the ELISA was used without positive EBL camel sera. However, 5 negative dromedary sera from CVRL animals were included.

### **Bacterial Disease**

**Leptospirosis (LI)-** For leptospirosis, 164 milking dromedaries which were randomly selected from the 1119 sera were examined with the microscopic agglutination test (MAT). For MAT, an initial serum dilution of 1:20 was done and live cultures of 20 leptospira cultures were used.

### **Parasitic Disease**

**Neosporosis (NC)** is caused by *Neospora caninum*. The cELISA was bought from VMRD Inc., USA. This cELISA detects antibodies to NC. The HRP labelled NC-specific monoclonal antibody is directed against the NC tachyzoite antigen and not against the animal species tested.

For the production of positive sera, 2 female 8 year-old dromedaries kept at CVRL, were vaccinated subcutaneously twice within a 3 week period. A cattle dose of 5 ml of the Neosporum vaccine Bovilis<sup>®</sup>, Neoguard was administered. Negative dromedary sera were obtained from 5 castrated dromedaries kept at CVRL.

### **Results**

Table 1 gives an overview of diseases tested as well as test details, manufacturers and their e-mail addresses. Table 2 summarises the serological results of all 8 camel diseases on 1119 serum samples from dairy dromedaries and their calves. For Leptospirosis, only 164 sera were tested.

All 1119 were negative for Enzootic Bovine Leukosis. Antibodies were found to Rift Valley Fever, Bluetongue, Equine Rhinitis A, Camelpox, Bovine Viral Diarrhoea, Leptospirosis and Neosporosis.

### **Discussion**

**Rift Valley Fever (RVF)** has been present on the African continent, since its discovery in Kenya in 1931. This arthropod-borne viral disease may be acute or peracute, but mild to inapparent infections also occur. It is a significant zoonotic disease which in humans may develop into an uncomplicated influenza-like illness but can also take the form of a

haemorrhagic disease. The Phlebovirus is transmitted by 23 mosquito species. Clinical disease is limited to domestic ruminants and humans. Strikingly, all of the RVF epizootics described to date have followed unusually severe rainy seasons because the efficacy of the virus is dependent on a large number of vectors involved in the transmission and viraemia in both animals and humans. RVF in ruminants is inapparent in non-pregnant adult animals, but in outbreaks, it is responsible for many abortions and high neonatal mortality. Globalisation of trade and changing weather patterns should be a concern for the future spread of RVF out of Africa (OIE, 2007). In 2000-2001 it had spread to Saudi Arabia and Yemen (Shoemaker *et al*, 2002), which were the first confirmed outbreaks of RVF among humans and livestock outside Africa.

Antibodies to RVFV were found in 3% to 45% of dromedaries in different East African countries (Wernery and Kaaden, 2002). However, at present

it is not clear if dromedaries develop clinical signs or abort during epidemics of RVF, although some scientists are almost certain that deaths and abortions occur in this species ([promed@promed.isid.harvard.edu](mailto:promed@promed.isid.harvard.edu) 6.7.07). During the Mauritanian 1998 RVF outbreak, IgG antibodies were detected in dromedaries but no virus was isolated (Nabeth *et al*, 2001). It is of great relevance to carry out proper investigations to elucidate the role of RVF in camelids due to the zoonotic potential of this disease.

Four (0.35%) of the 1119 serum samples were positive in the RVF inhibition ELISA which has, according to Paweska *et al* (2005), a 100% specificity and sensitivity for camelids. These 4 samples were from adult dairy dromedaries. None of the calves had seroconverted. The low incidence of RVF is not unexpected because the Arabian Peninsula experiences very little rainfall, unfavourable for the breeding of high numbers of mosquitoes. However, it should

**Table 1.** Test kit details for serological investigations of antibodies to 8 infectious diseases in dairy dromedaries.

Disease	Test details	Manufacturer	e-mail address
<b>Viral Diseases</b>			
1. Rift Valley Fever (RVF)	cELISA	BDSL, UK	mail@bdsl.uk.com
2. Bluetongue (BT)	cELISA	IDVet, France	idvet.info@id-vet.com
3. Equine Rhinitis A (ERA)	SNT	CVRL, Dubai	cvrl@cvrl.ae
4. Camelpox (CP)	SNT	CVRL, Dubai	cvrl@cvrl.ae
5. Bovine Viral Diarrhoea (BVD)	cELISA	Institut Pourquier, France	info@institut-pourquier.fr
6. Enzootic Bovine Leukosis (EBL)	cELISA	Institut Pourquier, France	info@institut-pourquier.fr
<b>Bacterial Disease</b>			
1. Leptospirosis (LI)	Microscopic Agglutination Test		Weybridge, UK
<b>Parasitic Disease</b>			
1. Neosporosis (NC)	cELISA	VMRD, Inc.	vmrd@vmrd.com

**Table 2.** Serological results of 1119 dromedary sera investigated for 8 infectious diseases.

Disease	Test details	Positive		Positive %		Positive Total	
		Dam	Calf	Dam	Calf	Dam/Calf	Total%
<b>Viral Diseases</b>							
1. Rift Valley Fever (RVF)	cELISA	4	0	0.7	0	4	0.35
2. Bluetongue (BT)	cELISA	209	26	36.1	4.8	235	21
3. Equine Rhinitis A (ERA)	SNT	35	29	6.5	5.1	64	5.7
4. Camelpox (CP)	SNT	460	283	85	49	743	66
5. Bovine Viral Diarrhoea (BVD)	cELISA	18	1	3.1	0.1	19	1.6
6. Enzootic Bovine Leukosis (EBL)	cELISA	0	0	0	0	0	0
<b>Bacterial Diseases</b>							
1. Leptospirosis (LI)	Microscopic Agglutination Test (MAT)	19	-	12	-	-	12
<b>Parasitic Disease</b>							
1. Neosporosis (NC)	cELISA	76	77	13.1	14.2	153	13.7

be kept in mind to test camels when imported from countries which regularly experience RVF outbreaks.

**Bluetongue (BT)** is an acute arthropod-borne viral infection of sheep, cattle and wild ruminants with extreme manifestation variability, not only between different ruminant species, but also between different breeds of sheep. The disease primarily affects sheep and has recently spread to northern Europe including England with severe consequences to the livestock industry. The serotype BTV-8, which is responsible for the current outbreak in north-west Europe, also causes clinical disease in cattle. There are no obvious geographical topographic boundaries that might halt the dramatic advance of BTV-8. Outbreaks of BT and epizootic haemorrhagic disease of deer (EHDD), another culicoides-borne pathogen are also occurring in North America. BT is not transmissible to humans. Twenty four serotypes of the virus are known. The BTV-8 currently ravaging Europe poses unique challenges and wide international threats in coming years because of its unexpected severe disease in cattle and other ruminants as well as the emergence of new vector species and the vertical transmission of the virus. Although reports of BTV seropositive NWC and OWC exist, there are really only 2 statements (Fowler, 1998; Henrich and Reinacher, 2007) of BT cases in a llama and alpaca associated with respiratory distress followed by abortion. BT antibodies were detected in Saudi Arabian dromedaries by Ostrowski (1999) and Al-Afaleq *et al* (2006), as well as in sheep (35%) and dromedaries in the UAE (5%) (CVRL Annual Report, 1998). Our ELISA result on 1119 dromedaries showed a seroprevalence of 21%. However, so far, no disease has been observed. It is not known which serotypes circulate on the Arabian Peninsula.

**Equine Rhinitis A virus (ERAV)**, formerly known as equine rhinovirus, has been responsible for relatively large outbreaks of acute respiratory illness in adult horse populations worldwide. ERAV is pathogenic for a broad range of other animal species including man (Plummer, 1963). ERAV does also infect dromedaries and, unlike horses where ERAV targets the upper respiratory tract, in dromedaries the target organ appears to be the genital tract (Wernery *et al*, 2008b). Camels abort at a gestation period between 6 and 8 months without showing any clinical signs. Placentation in *Camelidae* is diffuse epithelio-chorial, similar to that of the equine species, and this anatomical particularity may, in part, explain the susceptibility of dromedaries to an equine virus.

The SNT on 1119 dromedary sera revealed 64 reactors (5.7%) with 35 positive dams (6.5%) and 29

positive calves (5.1%). It is likely that positive calves have received their antibodies from the milk. ERAV should also be considered as a cause of abortions, but it is also worthwhile mentioning that many ERAVs are not cytopathogenic in cell culture and may be missed in disease investigations unless RT-PCR is used for diagnosis (Wernery *et al*, 2008b).

**Camelpox (CP)** is the most frequent infectious viral disease of the camel and therefore, the most widely reported. It occurs in the dromedary and bactrian camels and has also been experimentally induced in NWC. The disease occurs wherever camel husbandry is practiced, except in Australia. Epidemics occur in regular cycles dependent on the rainy season and relationship of the density of the insect population to the number of immune camels in the population. Animals that have recovered from infection appear to develop a lifelong immunity. Serological studies in different countries have revealed a high prevalence to the Camelpox virus (CaPV), some upto 100% (Wernery and Kaaden, 2002). Our investigations also revealed that 743 (66%) of the 1119 dromedaries tested had SNT antibodies to CaPV. It cannot be excluded that antibodies in calves may originate from maternal antibodies rather than from a previous virus infection. It is therefore, recommended to vaccinate all calves at the age between 6 - 10 months with the attenuated Camelpox vaccine 'Ducapox' as well as all SNT negative adult dromedaries, although it cannot be ruled out that some of them are immune despite being SNT negative. It is known that in orthopoxvirus infections, the cell-mediated immunity seems to protect animals from disease rather than circulating antibodies (Fenner *et al*, 1988).

**Bovine Virus Diarrhoea (BVD) and Mucosal Disease (MD)** are two different clinical disorders caused by the same virus. BVD is an acute cattle disease with a brief course and a low mortality, whereas, MD is a deadly disease of low morbidity associated with a persistent infection. The disease is an important cause of economic loss worldwide in dairy and beef cattle, and the disease has been described in camelids (Wernery and Kaaden, 2002). During the last few years, several cases have been reported in NWC. Evermann (2006) and Belknap *et al* (2000) proposed that BVD should be considered as a cause of death in young and old NWC. The clinical signs are associated with chronic weight loss, diarrhoea, abortion, stillbirth, and persistently infected NWC do also occur (Hemmingson *et al*, 2006). Hegazy *et al* (1998) state that the main cause of abortions in dromedaries is the BVDV, which can reach 50% in some herds. No BVDV abortions have

been reported from the Arabian peninsula. Several serological studies have also indicated that camelids are susceptible to infection with the BVDV using serum neutralisation test and ELISA. The incidence of BVD antibodies in both NWC and OWC varied between 0.5% - 50% in different countries (Wernery and Kaaden, 2002), including Saudi Arabia with 18% serological reactors (Al-Afaleq *et al*, 2006). Our investigations showed a prevalence of 1.6% in 1119 dromedaries with 3.1% in adult milking dromedaries. A small number of dromedaries in our investigation were also doubtful (1 out of 578 dams and 2 out of 541 calves). It was not possible to establish if the BVD-positive calf stemmed from a positive dam.

This is a prevalence lower than that described by Wernery and Wernery (1990) with 9% in dromedaries in the UAE.

Extensive studies are now necessary to elucidate the entire disease pattern in camelids through extensive field observations, laboratory studies and infection experiments.

**Enzootic Bovine Leukaemia Virus (EBLV)** is an oncogenic retrovirus infecting B lymphocytes in cattle. The infection may lead to a persistent lymphocytosis and, in some adult cattle, to the development of lymphosarcomas. There is an antibody response to infection, and they are found in both serum and milk. Lymphatic leukaemia has been diagnosed in several dromedaries in the UAE through haematology or necropsy. However, serological and blood transmission studies have not detected any evidence of a causative agent (Wernery and Kaaden, 2002). Kinne and Wernery (2006) believe that dromedary leukaemia cases are of sporadic nature and therefore, propose to name this disease sporadic adult camel leukosis (SACL). All 1119 serum samples were negative for EBLV antibodies with the commercial cEBL ELISA indicating again that EBL does not exist in dromedaries.

**Leptospirosis** occurs worldwide and *Leptospira* infections have been reported from OWC and NWC (Wernery and Kaaden, 2002). However, the clinical presentation of leptospirosis in OWC has not yet been described, and there are some doubts as to whether the camel is even susceptible to the disease, although Krepkogorskaya (1956) has isolated *Leptospira* from camel organs. Several reports exist about the seroprevalence of different serotypes, which range from 0.8 to 52%. Leptospire are present in tubules of mammalian kidneys and are excreted in urine, often for several months. Streams and ponds can be the source of infection as well as aerosols of urine in

cowsheds and milk from infected cows. Rodents and dogs serve as the most important epidemiological reservoir in intensive cattle husbandry. Within the genus *Leptospira*, only the species *L. interrogans* is of medical importance. All pathogenic leptospirae are included under this designation. Due to the varying antigenic structure, *L. interrogans* consists of at least 20 serogroups and approximately 180 serovars. A broad spectrum of manifestation, from no clinical signs to severe clinical signs, has been described in animals with abortions, neonatal mortality and infertility.

The MAT is the gold standard for identifying serological cases. Titres above 1:100 are considered positive. The test, which uses live leptospire as antigen is highly sensitive and serovar-specific. Only 164 sera of the total 1119 dromedaries were examined with the MAT due to the laborious nature of this test. The leptospira seroprevalence of 164 dairy dromedaries was 19 (12%). The following most important serovars were observed: *L. copenhageni* (15 out of 19 cases), *L. ballum* (1), *L. autumnalis* (1), *L. tarassovi* (1) and *L. javanica* (1). Seven dromedaries had titres equal or above 1:800, indicating a recent or active infection.

More research is necessary to elucidate the role of leptospirosis in camelids, and to find the source of infection. *L. copenhageni* is recognised as a rodent serovar.

**Neosporosis** is a protozoan disease caused by *Neospora (N) caninum*. This parasite was earlier confused with *Toxoplasma gondii*. *N. caninum* was first recognised in dogs, and is regarded as the most common cause of cattle abortions in the USA. It can also cause limb disorders in bovine calves. *N. caninum* is one of the most efficiently transplacentally-transmitted organisms in cattle. Cows of any age abort from 3 months gestation to term (Dubey, 2003). Dogs are both intermediate and definitive hosts, whereas cattle, sheep, horses, goats, deer, buffaloes, foxes, coyotes as well as camels are natural intermediate hosts (Dubey, 1999). New reports have emerged that abortion also occur in NWC (Serrano-Martínez *et al*, 2007). The authors reported abortions in llamas and alpacas caused by *N. caninum* and therefore suggest that Neosporosis should be included during differential diagnosis of abortions in NWC. *N. caninum* infections were confirmed by immunohistochemistry and PCR. Whether this parasite infects OWC has not yet been properly documented. Antibodies to *N. caninum* have been demonstrated in dromedary sera from Iran (Sadrebazzaz *et al*, 2006) and Egypt (Hilali *et al*, 1998), but no abortions were reported.

Our investigation showed a *N. caninum* seroprevalence of 153 (13.7%) out of total 1119 sera. Seventy-six (13.1%) dams and 77 calves (14.2%) were positive. This is an unexpected result which requires extensive studies of this disease in camelids.

### Conclusion

The aim of our investigation was two fold, first of all to analyse the seroprevalence of 17 infectious diseases in 1119 dromedaries used for commercial milk production, and secondly to evaluate commercial test kits for their suitability in dromedaries. For import, export and participation in shows mainly ruminant test kits are in use for camelids (Gunsner *et al*, 2004).

Our seroepidemiological investigations have shown that dromedaries kept on the Dubai Camel Dairy Farm (EICMP) are free of antibodies to Foot-and-Mouth Disease (FMD), Rinderpest (RP), Peste des Petits Ruminants (PPR) and Enzootic Bovine Leukosis (EBL).

Antibodies were found to West Nile Fever (WNF), Rift Valley Fever (RVF), Bluetongue (BT), Camel pox (CP), Equine Rhinitis A (ERA), Bovine Viral Diarrhoea (BVD), Brucellosis, Tuberculosis (Tb), Anaplasmosis (AG), Leptospirosis (LI),

**Table 3.** Summary of serological results of 1119 dairy dromedaries for 17 infectious diseases compared with clinical signs.

Disease Investigated	Antibody %	Clinical Signs
Foot-and-Mouth Disease	0	None
Rinderpest	0	None
Peste des Petits Ruminants	0	None
West Nile Fever	38	None
Rift Valley Fever	0.35	Abortion (?)
Blue Tongue	21	None
Bovine Viral Diarrhoea	1.6	Abortion, congenital deformities
Enzootic Bovine Leukosis	0	None
Camel pox	66	External and internal pustules
Equine Rhinitis A	5.7	Abortion
Tuberculosis	0.09	Granulomas
Brucellosis	1.9	Abortion, hygromas
Anaplasmosis	0.5	None
Leptospirosis	12	None
Trypanosomosis	3.5	Emaciation
Toxoplasmosis	33.6	None
Neosporosis	13.7	Abortion(?)

Trypanosomosis (Tryp), Toxoplasmosis (TG) and Neosporosis (NC).

Table 3 summarises the results from both investigations (Part I and Part II) and adds our current knowledge on clinical signs for each disease.

Our investigations show that cELISAs are reliable test kits for use in camelids. However, when indirect ELISAs are applied more research is necessary to minimise false positives and negatives.

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