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Short Communication

INVESTIGATION OF OCCURRENCE AND PERSISTENCE OF BRUCELLOSIS IN CHRONICALLY INFECTED DROMEDARY DAMS (*Camelus dromedarius*) AND THEIR CALVES

M. D. von Hieber, A. R. Thomas, R. Raghavan, G. Syriac, J. Christopher, B. Johnson, M. Joseph, S.V. Jose, S. Jose and U. Wernery

Central Veterinary Research Laboratory, P.O. Box 597, Dubai, U.A.E.

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In particular, the purchase of 746 dromedaries by the UAE in 2008 was the starting point of this epidemiological study. Latter dromedaries' country of origin was the Sudan and they were purchased to enlarge the dairy herd at the "Emirates Industries for Camel Milk Products" in the UAE. Upon first investigations during quarantine it was revealed that 234 (31.4 %) of above mentioned dromedaries were serologically positive for brucellosis in Rose Bengal Test (RBT) and Complement Fixation Test (CFT). Although still in quarantine, most of the affected dromedaries were mated and delivered their calves between October 2009 and March 2010.

The project work was initiated by a comparative study of 221 dromedary serum samples from the brucellosis infected herd in order to estimate the sensitivity of Rose Bengal Test and competitive ELISA (Von Hieber, 2010). It revealed a 10.8% higher sensitivity of cELISA (87.3% cELISA vs. 76.5% RBT). The cause for this finding was ascribed to the broader range of detectable immune globulin classes in cELISA and to the spectro - photometric test evaluation method, which is more precise than adspective evaluation. These findings showed the superiority of cELISA over RBT for the brucellosis detection in dromedary camels.

The main focus, however, was on investigating alterations in brucellosis serology in 118 dromedary dams of the above mentioned herd. Data were gathered over a time of two years. After purchase from Sudan in 2008, 88.1% (RBT) of the tested dams were positive in the initial investigation. After 18 months, 116 dams gave birth to living calves. At that time, 82.2% of the dromedary dams were found positive in RBT and 89.8% in cELISA. Six months later all dams were re – tested. The serological investigations revealed a significant higher decrease in sero - prevalence within six months after parturition, compared with the period of 18 months prior to parturition. The percentage of positive dams had then declined to 69.9% (RBT) and 82.5% (cELISA), respectively. In total, a decrease of brucellosis positive dams of 18.2 % (RBT) was observed over a period of 24 months, whereby 5.9% (RBT) decrease were observed in the first 18 months after purchase and further 12.3 % (RBT) decrease within 6 months after parturition (Fig 1).

The reason for the decline in positive dams after parturition is not clear, but presumably parturition and lactation influenced the immune system of dromedaries to an unknown extend. A plausible reason for the decrease of positive cases within two years was most probably the chronic state of brucellosis. It was not exactly known how long they have been infected since there were no data available of the time in Sudan. However, it can be assumed they got infected already several years before the purchase in 2008 and the disease had turned into a chronic state. It is known that in chronic courses of brucellosis, Brucella organisms can retreat into biological niches, mostly into lymph nodes, which would explain the decline in antibody levels.

An alarming observation was made observing 4.8% of the studied dams, whose serological status had changed undulatingly from positive to negative to positive throughout the two years of investigation. This showed that it is impossible to claim a negative status for once brucellosis positive animals. It is

SEND REPRINT REQUEST TO M.D. VON HIEBER email: danielavonhieber@aol.com; cvrl@cvrl.ae

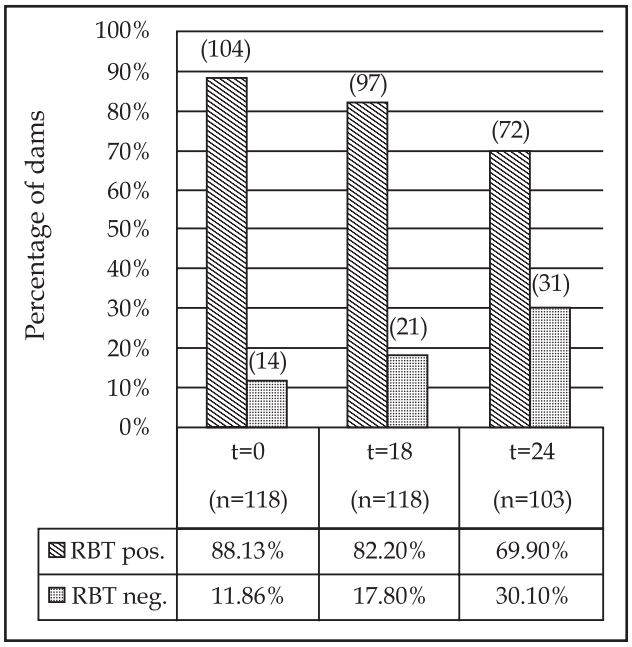

Fig 1. Comparison of RBT results (dams) over a period of 2 years.

Shown are the percentages of dams found positive (blue) versus negative (orange) in three investigations during 24 months. t=0 :time of purchase / first investigation; t=18 : time of parturition / after 18 months; t=24 : 6 months after parturition / after total. 24 months.

therefore recommendable, that for the eradication of brucellosis also those animals are euthanised in "stamping – out" programs, which have formerly been serologically and/or bacteriologicaly positive.

All calves were screened serologically for the first time within 24 hours after birth. This first investigation found 30.1% positive calves with RBT and 39.6% with cELISA. A second screening took place 6 months later. Then, most of the calves were found serologically negative. Only 1.1% (RBT) and 15.9% (cELISA) positive calves, respectively, were found at that time. Further elucidation of antibody development in cELISA of positive calves showed a significant decline in the levels of immuno globulins compared with the immuno globulin levels after birth. This is due to the continuous decrease in maternal antibody levels, which the calves had ingested with the colostrum after birth. Maternal antibodies in dromedary calves usually disappear until six to eight months post partum. Additional bacteriological blood cultures found no evidence of acute brucellosis infections. Therefore, calves of chronically infected dams seemed not to be at risk to contract an acute brucellosis infection. However, for confirmation of this finding further investigations of the calves, when adult and/or pregnant, are recommendable.

Since the cultivation of Brucella spp. has been previously reported to be tedious and difficult, several trials were performed to improve the cultivation frame work before starting with the investigations of the study. However, the main focus was on different culture media and less on culturing features. Two typical media, Brain Heart Infusion (BHI) and Brucella specific medium (BSM), were compared. BSM medium was based on Farrel's medium and supplemented with a range of antibiotics, to suppress growth of non – Brucella species. In this specification BSM has been served as the main culture medium for Brucella spp. in CVRL for 13 years. BHI medium supplemented with a range of antibiotics revealed its clear superiority over BSM in connection with the duration of incubation and the density of bacterial growth reached during incubation.

Along with bacteriological and serological investigations of the test herd, also tissue based rt – PCR was performed of placentas, lymph nodes, lung, liver and spleen, which were all negative. Due to these results, the sensitivity of rt – PCR was tested by using either spiked tissue samples with *B. melitensis* or dilutions of *B. melitensis* colonies in several different solvents. The results showed that the presence of a high amount of non – target DNA interferes with the efficiency of the PCR method. The tests emphasised the low sensitivity for the tissue – based rt – PCR, but have also shown the method's reliability in the amplification of pure target DNA in bacterial dilutions.

In summary, this study revealed that the initial comparison of RBT and cELISA has proofed the superiority of cELISA over RBT for serological investigations also in camels. Further research was initiated to include other serological test like CFT and STAT to find the most reliable test of the serological methods for brucellosis diagnosis [Gwida and El-Gohary (in press)].

The investigation of dromedary dams showed that a chronic state of brucellosis goes along with a very low abortion rate, no pathological alterations in organs and obviously no shedding of Brucella organisms through milk. Furthermore, the number of positive animals was steadily decreasing over the observed period of time. This seems to be caused by the retreat of Brucella organisms in biological niches, as it was already described elsewhere for the chronic state. The changes of the antibody development measured with cELISA underlined this finding: it was observed that even in positive dams the amount of antibodies, and thus the titres, declined over a period

of 6 months. This is a general observation and took also place during the 18 months prior to parturition. Prudence should be exercised in generally claiming dromedaries Brucella negative after only one negative serological result. Hiding Brucella organisms can be reactivated, reflected by re – emerging positive titres, such as observed in the test herd. Absolute certainty of eradicating camel brucellosis can only be achieved by "stamping – out" methods.

A special influence on the course of brucellosis in dromedaries can be attributed to the camel's very special immune system. Nanobodies, a 15 kDa big antibody of the IgG2 and IgG3 subclass, have the ability to penetrate in folds and crevices more effectively and subsequent neutralise antigens with more efficacy.

Concerning the role of milk as vehicle of transmission, more investigations shall be undertaken: in case dromedaries are also intermittently shedding animals such as cows, the probability of detecting bacteria with only two sampling events is low. For further clarification, an investigation of consecutively taken milk samples over a longer period of time is recommendable, including both, acute and chronic cases.

None of the investigated calves contracted an active infection during the period of this study. All serological positive calves had acquired the antibodies with colostrum after parturition. Maternal antibodies are supposed to disappear within the first six to eight months after parturition. This was also observed in the studied calves. It seemed that calves from positive mothers with chronic brucellosis did not contract the disease by close contact during parturition or by ingestion of milk. However, retesting the calves when they have matured or during their first pregnancies is recommendable to gain certainty on this matter.

References

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